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# Medical Technologist Exam

# SECRETS

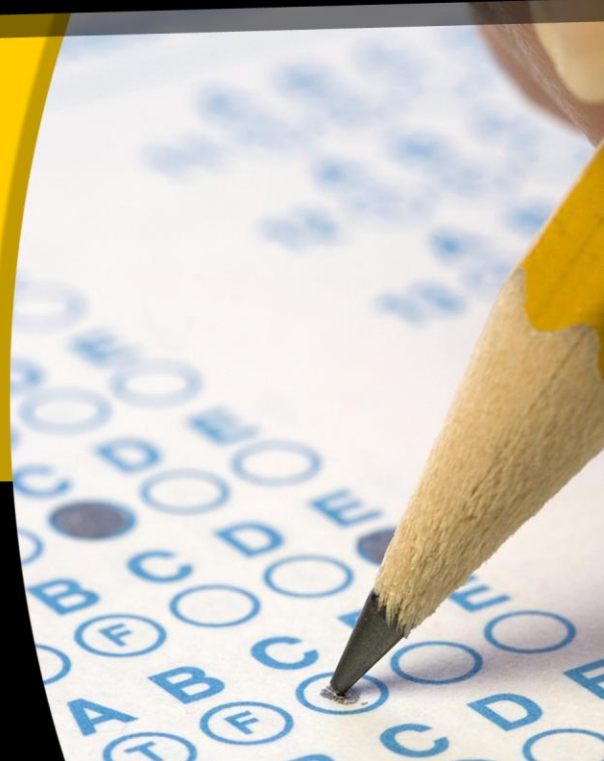
## Study Guide

Your Key to Exam Success

Effective,  
Affordable  
Help from the  
*World's Most  
Comprehensive  
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Company*

MT Test Review for the  
Medical Technologist Examination

Published by  
**Mometrix Media LLC**



Dear Future Exam Success Story:

Congratulations on your purchase of our study guide. Our goal in writing our study guide was to cover the content on the test, as well as provide insight into typical test taking mistakes and how to overcome them.

Standardized tests are a key component of being successful, which only increases the importance of doing well in the high-pressure high-stakes environment of test day. How well you do on this test will have a significant impact on your future- and we have the research and practical advice to help you execute on test day.

The product you're reading now is designed to exploit weaknesses in the test itself, and help you avoid the most common errors test takers frequently make.

### **How to use this study guide**

We don't want to waste your time. Our study guide is fast-paced and fluff-free. We suggest going through it a number of times, as repetition is an important part of learning new information and concepts.

First, read through the study guide completely to get a feel for the content and organization. Read the general success strategies first, and then proceed to the content sections. Each tip has been carefully selected for its effectiveness.

Second, read through the study guide again, and take notes in the margins and highlight those sections where you may have a particular weakness.

Finally, bring the manual with you on test day and study it before the exam begins.

### **Your success is our success**

We would be delighted to hear about your success. Send us an email and tell us your story. Thanks for your business and we wish you continued success-

Sincerely,

Mometrix Test Preparation Team

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# Top 20 Test Taking Tips

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1. Carefully follow all the test registration procedures
2. Know the test directions, duration, topics, question types, how many questions
3. Setup a flexible study schedule at least 3-4 weeks before test day
4. Study during the time of day you are most alert, relaxed, and stress free
5. Maximize your learning style; visual learner use visual study aids, auditory learner use auditory study aids
6. Focus on your weakest knowledge base
7. Find a study partner to review with and help clarify questions
8. Practice, practice, practice
9. Get a good night's sleep; don't try to cram the night before the test
10. Eat a well balanced meal
11. Know the exact physical location of the testing site; drive the route to the site prior to test day
12. Bring a set of ear plugs; the testing center could be noisy
13. Wear comfortable, loose fitting, layered clothing to the testing center; prepare for it to be either cold or hot during the test
14. Bring at least 2 current forms of ID to the testing center
15. Arrive to the test early; be prepared to wait and be patient
16. Eliminate the obviously wrong answer choices, then guess the first remaining choice
17. Pace yourself; don't rush, but keep working and move on if you get stuck
18. Maintain a positive attitude even if the test is going poorly
19. Keep your first answer unless you are positive it is wrong
20. Check your work, don't make a careless mistake

# Blood Bank

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## **Determining donor eligibility**

The following are explanations for determining if certain individuals would be able to donate whole blood at the present time:

- 21-year old woman who received a tattoo 14 months ago: She can donate whole blood at present; prospective donors are disqualified from giving blood within 12 months of receiving a tattoo because of hepatitis concerns
- 35-year old man who went on a trip to Nigeria 3 months ago: He cannot donate blood for one year because malaria is prevalent in Nigeria. If he was also born or lived in Nigeria, or had close contact with West Africans, then he is banned from ever donating because of Type O HIV.
- 33-year old woman with a hematocrit of 38%: She is allowed to donate whole blood because her hematocrit is within the normal range for an adult female of 38% to 46%; female anemia is a hematocrit of 36% or below. Also, her hemoglobin must be at least 12.5 g/dL to qualify.
- 48-year old man who received a blood transfusion 5 months ago: He must defer donation for one year after his transfusion in the USA, due to the possibility of hepatitis B, which can incubate six months. He cannot donate at all if he was transfused in the UK or West Africa.

## **Blood donor requirements and basic examinations**

Basic examinations and requirements of blood donors are:

- Temperature: lower than 99.5°F
- Blood pressure: at least 80/50 mm/Hg and no higher than 180/100 mm/Hg
- Pulse: between 50 and 100 bpm, and if you are paced, few irregular beats
- Body weight: at least 110 lbs
- Hematocrit: at least 39% for males and 36% for females
- Hemoglobin: at least 12.5 g/dL

## **Donor exclusion periods**

The following are exclusion periods for donors:

- Malaria: Wait three years after receiving antimalarials or living in a malaria-endemic country, and one year after travelling in a malaria-endemic country.
- Aspirin: 48 hours from last ingestion for platelet apheresis, but no deferral for whole blood donation
- Viral hepatitis: permanent exclusion
- Accutane use: one month after last use
- Body fluid exposure: one year following exposure, especially after jail detention, sex with a hepatitis carrier, blood transfusion, or a human bite

- Clotting factor injections: The health historian excludes all “bleeders” except Factor V deficiency (parahemophilia or Owren's disease) who do not take anticoagulants
- Male prospective donor who had even one homosexual contact since 1977:  
permanent exclusion

### **Autologous donation**

Autologous blood donation means the Blood Bank saves units of the patient's own blood for his/her exclusive personal use. *Allogeneic* blood donation means the patient receives blood from a donor. Providing no clerical error or freezer failure occurs, it is safer to receive an autologous transfusion than an allogeneic transfusion. A proper autologous donation means there is no delay for cross-matching, no possibility of transfusion reaction, and no disease transmission. However, autologous donation is expensive, time-consuming, and sometimes results in hypovolemia or anemia. Unnecessary autologous transfusion causes volume overload. The five kinds of autologous donation are: (1) preoperative autologous blood donation (PABD); (2) intraoperative blood salvage; (3) intraoperative hemodilution; (4) postoperative blood salvage; and (5) autologous self-stored blood banking.

### **Transfusion procedure and reactions**

Always double-check the tag on the blood bag against the Blood Bank requisition *before* you release the unit to the nurse for transfusion. During the blood transfusion, check *all* of your patient's vital signs every 15 minutes, including body temperature, pulse, blood pressure, and respiration. If you observe any of these signs, then *stop* the transfusion immediately: hives; trembling (rigors); vomiting; flushed face; clammy skin; difficulty breathing (dyspnea); bloody urine (hematuria); fever; weak and rapid pulse; low blood pressure; or yellow jaundice. Ask the patient if he/she feels itching, lower back pain, nausea, anxiety, or chills. Keep the vein open with normal saline. Notify the attending physician, the patient's nurse, and the Blood Bank manager. Take the blood bag back to the lab for investigation. Do not transfuse another unit until the Charge Technologist confirms the cause of the reaction and rectifies it. If your patient is hemorrhaging, the doctor can safely order O- blood (universal donor).

Ensure the patient has an IV in place with saline running to keep the vein open before you release blood for transfusion. Confirm that the doctor's order and patient's or guardian's signed consent form are on file. Match the patient's arm band to the requisition and firmly attached blood unit label. Visually inspect the unit for hemolysis, lipemia, and foreign bodies. Release only one unit at a time, unless it is a severe trauma.

### **Storage and transportation**

Store red blood cells that have never been frozen between 1°C and 6°C (34°F to 46°F). Set an alarm on the refrigerator to sound if this temperature range is exceeded. Log the temperature at least once every four hours. If CPD (citrate-phosphate-dextrose) or CP2D

(citrate-phosphate-2-dextrose) is the anticoagulant, then the expiration date for the red blood cells is three weeks after collection. However, if the anticoagulant is CPDA-1 (citrate-phosphate-dextrose-adenine-one), red blood cells can last five weeks. If the anticoagulants AS-1, AS-2, or AS-3 were used, then the red blood cells will last six weeks after collection. Flush expired or incorrectly stored blood down the Dirty Utility sink or latrine, and discard the bag in a biohazard garbage.

Transport platelets at room temperature and avoid hemolysis by jostling. Transport red blood cells between 1°C and 10°C; place red blood cells in a Styrofoam box, resting on an ice bag, inside a cardboard box. Ship frozen blood components wrapped well on dry ice.

The ideal storage conditions and usual shelf life of blood products are:

- Frozen red blood cells: -65°C or less; 10 years from collection date
- Platelets: between 20°C and 24°C; five days from collection date
- Cryoprecipitate: -18°C or less; one year after collection date
- Pooled platelets: between 20°C and 24°C; four hours after pooling
- Thawed red blood cells: between 1°C and 6°C; 24 hours after thawing

## **Blood product use**

Transfusing whole blood is wasteful. Usually, whole blood is only necessary for massive traumas where the patient loses 25% of his/her blood supply. Lab technicians in the developed world separate whole blood into red blood cells, platelets, and plasma with a centrifuge. One unit of whole blood can be split into blood products to conserve blood for these common situations:

- Washed red blood cells: infant and intrauterine transfusions; IgA deficiency with anti-IgA antibodies; previous anaphylaxis, allergic, or febrile reactions to donated plasma proteins
- Leukocyte-reduced red blood cells: frequently transfused chronic patients
- Irradiated red blood cells: bone marrow and progenitor cell transplants; cancer chemotherapy or radiation therapy; intrauterine transfusions; occasionally given to immunodeficient individuals or premature infants

## **Blood components**

The methods for removing components from the patient's whole blood are:

- Plasmapheresis: a form of hemapheresis that removes only plasma from the donor's blood, and the nurse returns the remaining blood products to the donor, who may undergo this process once every eight weeks
- Plateletpheresis: only platelets are removed from the donor's blood with an electronic apheresis instrument; donors may undergo this process once every 48 hours
- Leukapheresis: only white blood cells are removed from the donor's blood with an electronic apheresis instrument; donors may undergo this process no more than twice a week or 24 times in one year

## Blood separation process

Centrifuge one 450 mL bag of whole blood at slow speed for separation into packed red blood cells (PRBCs) and platelet rich/concentrated plasma. Bag the PRBCs and mix with additives. Bag the platelet rich plasma separately in a platelet pack. Repeat centrifugation of the platelet rich plasma only at high speed to obtain one unit of random donor platelets and one unit of fresh frozen plasma (FFP). Put the plasma into a fresh frozen plasma bag; leave the platelets in their original container.

## Indications

- Red blood cells: use after massive trauma; before surgery when the patient is anemic; preradiation or prechemotherapy; for sickle cell anemia; for premature infants
- Platelets: use to treat excessive post-operative bleeding; during chemotherapy; after a bone marrow transplant
- Plasma: use for liver disease coupled with bleeding; abnormal coagulation reaction after transfusion; before surgery for those already taking anticoagulant drugs
- Whole blood: use when a trauma patient loses 25% or more of the entire blood volume; exchange transfusions

## Erythroblastosis fetalis

*Erythroblastosis fetalis* is a potentially lethal condition, also known as hemolytic disease of the newborn (HDN). The pregnant mother has a different blood type than her unborn infant. The problem may be either Rh or ABO incompatibility. The mother's IgG antibodies travel through the placenta and attack the red blood cells of her fetus. The fetus may become anemic, leading to heart failure. Alternatively, hemoglobin from destroyed red blood cells is converted into indirect bilirubin, leading to jaundice, retardation, brain damage, deafness, and sometimes perinatal death. The baby is edematous, with hydrops and a swollen liver or spleen.

The most severe, but least common, form of HDN is Rh incompatibility. A D- mother develops D antibodies in response to a D+ baby. If the same mother subsequently has another D+ fetus, then the D antibodies from her first pregnancy attack the red blood cells of the second fetus. The delivery team performs an exchange transfusion, aggressive hydration, and phototherapy to reduce the complications of HDN, which are neurological syndrome, kernicterus, *hydrops fetalis*, and hypotonia. Rh incompatibility HDN is preventable if the mother receives injections of RhoGAM immune globulins.

ABO hemolytic disease is a less severe, but more common, form of HDN. A type A mother reacts to a type B or AB fetus; or a type B mother reacts to a type A or AB fetus; or a type O mother reacts to an A, B, or AB fetus. ABO hemolytic disease is treated with phototherapy, to remove excess bilirubin.



To prevent HDN, the obstetrician treats the mother with RhoGAM, not the fetus or father. The standard dosage is as follows:

- A pregnant D- mother receives 300 micrograms (1,500 IU) of RhoGAM IgG anti-D (anti-Rh immune globulin) intramuscularly between 26 and 28 weeks of gestation, and another 300 micrograms within 72 hours after delivery
- A D- woman who had an abortion, amniocentesis, percutaneous umbilical blood sampling, abdominal trauma, intrauterine transfusion, or ectopic pregnancy receives 50 micrograms (250 IU) of MICRhoGAM within 72 hours of the procedure

### **Kleihauer-Betke acid elution test**

Perform a Kleihauer-Betke (KB) test after a birth to determine the percentage of sensitizing fetal cells mixed with the mother's blood, so the doctor can calculate the correct dosage of RhoGAM. First, take a blood smear from the mother. Dip it in an acid buffer. Apply a counterstain. The mother's blood cells are bleached pale. The fetal cells stain bright pink and are 1.22 times the size of the mother's cells. A normal adult has less than 0.01% of fetal hemoglobin (Hb F) present. A normal, full-term newborn has more than 90% Hb F present. The percentage of fetal cells is equal to the number of fetal cells counted, divided by the total number of cells counted; multiply the percent of fetal cells by 50 to derive the milliliters of fetal blood present in the mother's circulation.

### **DAT**

The direct antiglobulin test (DAT) is also called the Direct Coombs test. DAT detects hemolytic disease of the newborn, mismatched organ transplants, autoimmune hemolytic anemia, or transfusion reaction. DAT tells the doctor if the patient's red blood cells are coated with IgG antibodies or complement proteins. Obtain a heel prick from the infant, or a lavender or red stoppered venipuncture tube from the adult. Put two drops of blood in a clean test tube. Wash the red blood cells three times with isotonic saline solution. Make a 3% suspension of washed cells. Put one drop of washed cells into a tube marked POLY IS and a tube marked POLY 5". Wash the tubes again with the cell button on top. Wipe dry with a tissue. Add one drop of Coombs serum (polyspecific antihuman globulin) to each tube. Mix well. Incubate the POLY 5" tube at room temperature for five minutes. Centrifuge the POLY IS tube. Resuspend its contents. Observe for agglutination, which indicates either complement proteins or IgG antibodies are present. If the POLY IS tube is negative, look at the blood for microscopic agglutination. If the blood is not agglutinated, add one drop of IgG-coated Coombs Control Cells to the POLY IS tube and centrifuge it. If the POLY IS tube still did not agglutinate, then the test is invalid. Test the POLY 5" tube. If the POLY IS tube is positive, do not test the POLY 5" tube.

## **D-L test**

The Donath-Landsteiner test diagnoses a rare autoimmune hemolytic anemia, paroxysmal cold hemoglobinuria (PCH), also called Donath-Landsteiner syndrome (DLHA). Three people in 100,000 are affected. Usually, syphilis precedes PCH, but it also occurs a week after a viral upper respiratory infection or bacterial pneumonia. PCH can be idiopathic, or associated with non-Hodgkin lymphoma, or oat cell carcinoma. PCH is most common in male children.

D-L autoantibody is a cold-reacting polyclonal IgG antibody that attacks P antigen on red blood cells. A PCH patient must avoid moving from warm to cold. Temperatures less than 30°C trigger D-L autoantibody to activate complement, which hemolyzes blood. PCH damages the patient's kidneys, hands, and feet. PCH causes anemia, and sometimes cardiac failure. Signs and symptoms are: bloody urine; back, abdominal, leg, and head pain; chills; fever; cyanosis; pallor; jaundice; tachycardia; dyspnea; hives; nausea; vomiting; and malaise. If it does not resolve spontaneously, PCH requires a blood transfusion.

To perform a Donath-Landsteiner test: Warm two red stoppered venipuncture tubes to 37°C. Draw blood from your patient. Allow one tube to clot at 37°C; this is the control. Place the other tube on ice (4°C) for one hour, then warm it to 37°C for 30 minutes. Centrifuge both tubes at 37°C. Observe the serum for hemolysis. The control serum should be clear. Pink serum in the previously chilled tube is a positive D-L result.

## **Donor reactions**

Very few blood donors experience an adverse reaction (0.28% to 0.6%). However, your donor may develop nausea, dizziness, or syncope, especially if you are collecting whole blood or he/she is sensitive to citrate for apheresis. Observe your patient for agitation, pallor, bruising, sweating, and hyperpnea. The change in blood pressure during collection may trigger a seizure, heart trouble, or incontinence in susceptible patients. Stop the collection immediately. Remove the tourniquet. Seal the blood collection bag. Place the patient in recovery position. Ensure his/her airway is open. Check his/her pulse rate. If your patient faints, try smelling salts. If your patient is alert, give a sugary drink. Apply a cold compress to your patient's forehead if he/she wishes. Keep your patient reclining for at least 20 minutes, and assist him/her to stand.

## Agglutination reactions

The six grades of agglutination reaction for red blood cells are:

- Zero: lowest grade; no agglutinative red blood cells are present
- +w: red blood cell button divides into almost invisible or invisible clumps
- 1+: red blood cell button divides into a number of small and medium-sized clumps
- 2+: red blood cell button divides into numerous medium-sized clumps
- 3+: red blood cell button divides into large clumps
- 4+: red blood cell button does not break into clumps; free red blood cells cannot be seen in the background

## Transfusion reactions

Only 0.004 blood recipients per 1,000 have an adverse transfusion reaction. The types of transfusion reaction are: acute; delayed; immune-mediated nonhemolytic; anaphylactic; nonhemolytic febrile reaction; transfusion-related acute lung injury; graft-versus-host disease; massive transfusion complications; and acquired diseases.

### Acute

A recipient experiences an acute hemolytic transfusion reaction immediately after a blood transfusion. Signs and symptoms are: fever; chills; fast heart beat (tachycardia); blood clotting (DIC); hemoglobinemia; bloody urine (hemoglobinuria); low blood pressure (hypotension); kidney failure; and cardiac collapse. Acute hemolytic transfusion reaction results from an incompatible transfusion, with potentially deadly reactions between antibodies and antigens, and is usually traced to a clerical error. The laboratory indications that your patient has an acute transfusion reaction are decreased haptoglobin, elevated bilirubin, and elevated plasma free hemoglobin.

### Delayed

The recipient typically experiences a delayed hemolytic transfusion reaction five to seven days after a transfusion. Signs are fever or mild jaundice. Delayed transfusion reaction is slightly more common than acute hemolytic transfusion reaction. However, delayed reaction does not usually pose a threat to survival. The following laboratory tests indicate a possible delayed hemolytic transfusion reaction: positive antibody screen post-transfusion; positive direct antiglobulin test; decreased hematocrit and hemoglobin.

### Immune-mediated nonhemolytic transfusion reaction

Immune-mediated nonhemolytic transfusion reaction means the recipient has HLA antibodies that react to the donor's antigens and cytokines. It is especially common in multigravida women (multiple pregnancies) or recipients of multiple transfusions. Signs and symptoms are back pain, headache, nausea, vomiting, and a fever beginning up to 24 hours after the transfusion. It is the most common type of transfusion reaction.

### Anaphylactic transfusion reaction

Life-threatening anaphylaxis begins almost immediately after a transfusion is initiated, from a severe allergic reaction. Signs are bronchospasms, wheezing, and cough, but not fever. Anaphylactic transfusion reaction is fatal if not treated immediately. Usually, the problem is that your patient inherited an immunoglobulin A (IgA) deficiency. Complement-binding anti-IgA antibodies form when your patient is exposed to the donor's IgA. However, proteins in the donor's plasma could also trigger a minor allergic reaction, and this is especially likely with fresh frozen plasma (FFP), pooled platelets, and whole blood.

### Nonhemolytic febrile reaction

Cytokines form when blood is stored. Your patient may develop a fever as a reaction to the donor's cytokines, but it is unlikely that he/she will go on to develop breathing problems (respiratory distress) or low blood pressure (hypotension). The fever will probably be self-limiting, but you must still notify the doctor, nurse, and lab manager, as a precaution.

### Transfusion-related acute lung injury (TRALI)

Any transfused blood product that contains plasma can harm your patient's lungs. The usual suspects are fresh frozen plasma (FFP), whole blood, and packed red blood cells (pRBCs). Rarely, cryoprecipitate, granulocytes, concentrated platelets, and apheresis platelets trigger TRALI. The donor's antileukocyte antibodies attack the recipient's white blood cells, which activates complement. (The culprits may be granulocyte or HLA class I or HLA class II antibodies in the plasma of female donors who had multiple pregnancies.) Mild symptoms start in one or two hours and are full-blown by four to six hours. Blood vessels in your patient's lungs become more permeable and leak, so your patient develops pulmonary edema, cyanosis, hypoxia, and dyspnea. Look for fever, chills, and hypotension. Your patient needs supplementary oxygen and perhaps mechanical ventilation. The doctor will order arterial blood gases and a chest x-ray. The radiologist will report bilateral pulmonary infiltrates. TRALI is the third most common cause of death by transfusion reaction.

### Graft-versus-host disease (GVHD)

Normally, the recipient's immune system recognizes the donor's lymphocytes are foreign, and destroys them. However, if the donor is immunocompromised, or if the recipient's HLA haplotype is heterozygous and the donor's is homozygous, then the donor's lymphocytes are not killed and GVHD results. Graft-versus-host disease means the recipient's lymphatic tissue is attacked by the donor's lymphocytes. GVHD affects mostly babies, elderly, and cancer and transplant patients. Consider giving them irradiated blood to prevent GVHD. The mildest form of GVHD is Grade I and the most severe is Grade IV. Symptoms typically emerge three to 30 days after transfusion or organ transplant, but can take more than 100 days to appear. Watch for: hives; fever; diarrhea; sloughing skin; chronic cough; irritated mouth and eyes; muscle cramps; and abnormal liver function. Treatment is cyclosporin, methotrexate, prednisone, and removal of the transplanted organ (graft). Untreated Grade IV GVHD results in death from sepsis.

### Massive transfusion complications

When your trauma patient has more than half of his/her blood supply replaced in one day, or receives 10 units of blood in a few hours, expect massive transfusion complications. Your patient will likely develop volume overload, hypothermia, coagulopathy, and calcium and potassium fluctuations. Complications may progress to metabolic alkalosis, heart and liver failure.

### Acquired diseases

Many bacterial, viral, and prion diseases are transmitted through blood transfusion, including: hepatitis; HIV; HTLV-1; babesiosis; CJD; TTV; anaplasmosis; cryoglobulinemia; Leishmaniasis; Chagas disease; Lyme disease; and toxoplasmosis.

### **Compatibility**

Five million Americans receive blood transfusions annually. After disease and medication screening, the technician performs Landsteiner tests for group and type to prevent agglutination reactions. The Landsteiner system has been in use since 1901, and looks for inherited antigen proteins on red blood cells, and antibodies in blood plasma:

- AB+ donates to AB+, but is the *universal receiver* who tolerates AB+, AB-, A+, A-, B+, B-, O+, and O-.
- AB- donates to AB- or AB+, but receives AB-, A-, B-, or O-.
- A+ donates to A+ or AB+, but receives A+, A-, O+ or O-.
- A- donates to A-, A+, AB-, or AB+, but receives from A- or O-.
- B+ donates to B+ or AB+, but receives from B+, B-, O+ or O-.
- B- donates to B-, B+, AB-, or AB+, but receives from B- or O-.
- O+ donates to O+, A+, B+, or AB+, but receives from O+ or O-.
- O- is the *universal donor* who donates to AB+, AB-, A+, A-, B+, B-, O+, or O-, but receives O-.

## Ethnicity and genotype

If the recipient is frail, then the donor's ethnicity may be important. For example, to reduce the likelihood of a transfusion reaction in a fragile child recipient with sickle cell anemia, the hematologist may prefer a donation from an African-American, if one is readily available. According to the American Red Cross, the blood type frequencies for Americans by ethnicity are as follows:

<i>Blood Type</i>	<i>White</i>	<i>Black</i>	<i>Latino</i>	<i>Asian</i>
O+	37%	47%	53%	39%
O-	8%	4%	4%	1%
A+	33%	24%	29%	27%
A-	7%	2%	2%	0.5%
B+	9%	18%	9%	25%
B-	2%	1%	1%	0.4%
AB+	3%	4%	2%	7%
AB-	1%	0.3%	0.2%	0.1%

ABO alone provides insufficient evidence to prove a child's parentage. Generally, the child inherits his/her blood genotype from the parents in the pattern below. A black square indicates an impossible result, and an x indicates a possible result:

<i>Child's Possible Blood Inheritance</i>	<i>Parents' Landsteiner Blood Group Combinations</i>									
	AB AB	AB B	AB A	AB O	B B	A B	A A	O B	O A	O O
O	■	■	■	■	x	x	x	x	x	x
A	x	x	x	x	■	x	x	■	x	■
B	x	x	x	x	x	x	■	x	■	■
AB	x	x	x	■	■	x	■	■	■	■

The University of Arizona's Biology Project offers a very handy, free blood genotype calculator for you to practice with online, at

[http://www.biology.arizona.edu/human\\_bio/problem\\_sets/blood\\_types/inherited.html](http://www.biology.arizona.edu/human_bio/problem_sets/blood_types/inherited.html)

However, remember that ABO and Rh factor (D antigen) are not the only tests required for paternity suits. The MT also tests the mother, child, and suspected father for C, c, E, and e, and H antigens. (About 1 in 10,000 people of East Indian or Taiwanese descent have the

potentially misleading Bombay phenotype, also known as Oh or h/h blood type.) The attending physician and the genetics counselor are the appropriate staff members to discuss paternity with the patient, rather than the MT.

## Alternative systems

The International Society of Blood Transfusion (ISBT) recognizes 26 blood classification systems and eight antigen collections. Landsteiner's ABO and Rh is the most prevalent blood classification system, but the MT must also be conversant with at least the following systems and their abbreviations:

- *Diego*: antigens Di<sup>a</sup>, Di<sup>b</sup>, and Wr<sup>a</sup> are the most significant, but there are 21 in all, with either IgG or IgM antibodies. Implicated in moderate to severe delayed transfusion reactions and mild to severe hemolytic disease of the newborn.
- *Kell*: antigens K (Kell), k (Cellano), Kpa and Kpb, usually with IgG antibodies; IgM is uncommon. Kell immunization causes severe fetal anemia by suppressing the baby's red cell production. K<sub>0</sub> is the null phenotype, associated with chronic granulomatous disease. Anti-Ku produces a potentially fatal transfusion reaction, so if your patient is K<sub>0</sub>, only transfuse K<sub>0</sub> blood products.
- *Kidd*: antigens Jk1, Jk2, and Jk3; usually IgG antibodies, but occasionally IgM antibodies. Kidd antibodies are difficult to detect when performing a routine cross-match. Implicated in severe, delayed hemolytic transfusion reactions and mild hemolytic disease of the newborn.
- *Duffy*: antigens Fy<sup>a</sup>, Fy<sup>b</sup>, Fy3, Fy4, Fy5, and Fy6, with almost exclusively IgG antibodies. IgM is uncommon. Associated with mild hemolytic disease of the newborn, mild to severe transfusion reactions, and sickle cell anemia. Absence of Duffy antigens confers some resistance to malaria, and is most common among people of West African descent.
- *Lutheran*: antigens Lua and Lub with IgG antibodies. Use a Western Blot or Flow Cytometry. Lutheran antibodies are not implicated in transfusion reactions, and rarely cause mild hemolytic disease of the newborn.
- *Lewis*: antigens Le a and Le b are formed in plasma and absorbed by RBCs. Almost exclusively IgM antibodies activate complement. Perform the Indirect Antiglobulin Test to detect them. Lewis antibodies are implicated in mild (subclinical) hemolytic disease of the newborn.
- *MNS*: antigens M, N, S, and s (43 antigens in all), with IgM antibodies; anti-S and anti-s are IgG antibodies from pregnancy or blood transfusions. Implicated in delayed transfusion reactions, very rare hemolytic disease of the newborn, and antigen receptors of malarial parasites.
- *P*: P, P1, and LKE with almost exclusively IgM antibodies. Anti-P antibody is detected via a Donath-Landsteiner test for paroxysmal cold hemoglobinuria.

ISBT promotes a worldwide *numeric* blood nomenclature system to reduce confusion in Blood Bank. In March 2011, ISBT updated its standard terminology, which is available for free download at <http://iccbba.org/home>.

## Indirect Antiglobulin Test

The IAT looks for a weak expression of D antigen in blood. The MT performs an IAT for these patients only: (1) a D- baby born to a D- mother; (2) a BMT donor; (3) a father, when the obstetrician wants to avoid unnecessary RhIG injections.

Prepare a 2% to 4% suspension of washed red cells from the patient, and suspends them in saline. Label one test tube "D CONTROL" and the patient's surname, and inserts one drop of control sera into it. Label another test tube "IAT-D" and the patient's surname, and inserts one drop of Novaclone anti-D into it. Add one drop of the patient's cell suspension into each of the tubes. Check that the volume is correct. Mix both tubes. Incubate them at body temperature (37°C) for 15 minutes. Wash the cells with saline. Add two drops of anti-IgG into each tube. Mix to resuspend the red cell buttons. Centrifuge. Resuspend the buttons. Observe for agglutination with the naked eye only; do not look for microscopic agglutination. Grade and record the reactions as follows:

<i>Anti-D IAT</i>	<i>Control</i>	<i>Report</i>
No agglutination	No agglutination	D-
Agglutination	No agglutination	D+
Agglutination	Agglutination	Unable to interpret
Mixed field agglutination	No agglutination or agglutination	

If the result is negative, confirm by adding one drop of control cells, mix, resuspend, and observing for agglutination.

## Antibody enhancer

An antibody enhancer is the chemical that stimulates the formation of antigen/antibody complexes. For instance, the proteolytic enzymes papain, ficin, and bromelain are frequently used as antibody enhancers, because they increase red blood cell agglutination. By contrast, bovine albumin encourages sensitized red blood cells to form agglutination lattices. Low ionic strength solution (LISS) is often used to stimulate the formation of antigen-antibody complexes.

## Sensitization

Many in vitro antigen/antibody reactions start with sensitization, the point at which the antibody attaches to the antigen, but has not yet produced any agglutination or hemolysis. The optimal pH for sensitization is 7. The degree of sensitization depends on the incubation time, defined as the amount of time in which the antibody has to attach to the antigens. Also, antibodies react most strongly at body temperature (37°C). Finally, sensitization increases in proportion to the ratio of serum to cells; more serum means more available antibodies.



## Limitations of cross-matching

If the screening for disease, medication, and ABO Rh were satisfactory, then the MT proceeds with a cross-match. Remember that *in vitro* reactions (in the test tube) do not exactly predict *in vivo* reactions (in the body). Cross-matching does not detect viruses, bacteria, or parasites in donated blood. Patients can suffer delayed transfusion reactions or allergic reactions to proteins in the donor's plasma, neither of which can be predicted with cross-matching. Also, cross-matching has no effect on antibody formation in response to any foreign antigens in the red blood cells of the donor. For these reasons, cross-matching is not a foolproof way to make transfusion safe. The types of cross-matching are:

- Major cross match: required test before a transfusion; donor cells are tested with the serum of the potential recipient; antibodies come from serum, antigens come from donor cells.
- Compatible cross match: the MT ensures the mixture of potential recipient's and potential donor's blood produced neither hemolysis nor agglutination of cells *in vitro*.
- Incompatible cross match: the MT excludes potential donor blood that produced hemolysis or agglutination when mixed with the potential recipient's blood *in vitro*.

## Bacterial contamination of blood

Occasionally, blood products in storage suffer bacterial contamination, usually with *Yersinia enterocolitica* or *Pseudomonas fluorescens*. Blood cultures are not part of a cross-match. Suspect contamination if your patient develops diarrhea, fever, respiratory distress, and sepsis. The CDC usually traces contamination to the donor's skin or to poor bagging technique.

# Laboratory Practice

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## Safety acronyms

The following acronyms regarding safety:

- *CAP*: College of American Pathologists.
- *CBRN*: Chemical, biological, radiation, nuclear; part of emergency preparedness training in case of bioterrorism.
- *CDC*: Centers for Disease Control and Prevention in Atlanta encourages lab safety, monitors emerging diseases, and publishes reliable health information.
- *CLIA*: Clinical Laboratory Improvement Amendments (1988) ensures “accuracy, reliability, and timeliness of patient test results”. The U.S. Food and Drug Administration oversee CLIA.
- *DOT*: U.S. Department of Transportation
- *EEOC*: U.S. Equal Employment Opportunity Commission deals with discrimination and harassment.
- *HHS*: United States Department of Health and Human Services
- *HIPAA*: Health Insurance Portability and Accountability Act (1996) protects workers when they change or lose jobs by providing health insurance and privacy standards.
- *IATA*: International Air Transport Association sets shipping standards for road, rail, air, and water.
- *IMS*: Incident Management System controls CBRN events and how local authorities interact with labs.
- *JCAHO*: Joint Commission on Accreditation of Healthcare Organizations offers accreditation, certification, education, standards, and reports on patient safety.
- *OSHA*: U.S. Department of Labor’s Occupational Safety & Health Administration
- *PPE*: Personal Protective equipment

## Just-in-time inventory

Just-in-time inventory is an idea adopted from big car manufacturers, where supplies are not stockpiled, but are ordered from the vendor for delivery just a day or two before they are needed. This means your employer does not have to pay for expensive warehouse space – the vendor does – which helps with the lab’s cash flow. The Chemical Hygiene Officer will have no trouble listing what chemicals are kept on the premises, which OSHA requires. However, just-in-time makes it hard to figure out how much of a chemical your lab uses on a monthly basis, and often the danger depends on the level of exposure. OSHA does not require you to keep data on solutions with less than 1% of a harmful chemical or pre-packaged kit containing harmful chemicals. A useful chemical safety data sheet (MSDS) contains this information: Chemical name, trade name, hazard class; where and how it

should be stored; storage quantity; vendor's contact information; and vendor's catalog number.

## Chemical grades

Chemical grades, from highest to lowest purity:

- *ACS*: Highest purity grade. The only universal standard. Meets American Chemical Society benchmarks.
- *USB*: For reference materials because they have smaller amounts of contaminants or higher assay limits than reagent grade.
- *MB*: Low levels of trace metals and genetic material for molecular biology.
- *AR*: Analytical reagent grade for in-house lab work. Usually as pure as ACS, but lost certification because it was repackaged.
- *USP*: Meets United States Pharmacopeia potency standards for drug use, suitable for most lab work.
- *Lab grade*: Intermediate purity and low cost for teaching.
- *NF*: Meets National Formulary requirements, and contains moderate amounts of impurities.
- *Practical grade*: No official standards. Good quality for general use.
- *Pure*: Low-intermediate grade inorganic chemicals with no standards. Used for teaching because of impurities.
- *Chemically Pure (CP)*: For industrial use, not intended for use in food, but more pure than technical grade.
- *Technical or commercial grade*: Good quality, but impure. Industrial use only, not experimental or lab work.
- *Pyrotechnic*: Low quality for fireworks and industrial use.

## Sustaining a corrosive burn

Flooding corrosive powder burns with water will activate the chemical and deepen the burn. Take a piece of paper and gently brush off as much of the dry chemical as possible into a garbage can before diluting it with water. If it is liquid corrosive or in the eye, flood with cool, running water immediately for at least 15 minutes at an eyewash station or sink. Immerse if skin is sloughing. Visit a doctor immediately. If you cannot immediately consult a doctor, use the neutralizing agent supplied by your Chemical Hygiene Officer. If clothing is melted into the flesh, do not remove it. Recognize the severity to complete the incident report in 24 hours: First degree burns involve the top layer of skin and are red. Second degree burns are red, blistered, and swollen, with severe pain and involve the first two layers of skin. Third degree burns are charred black, red, yellow, or white and are leathery, with destroyed nerves and blood vessels and minimal pain.

## Safety concerns

### Waste disposal

Disposing of concentrated, infected human waste incorrectly infects the water and food supplies with diseases like cholera, dysentery, polio, and typhoid. Segregate contaminated solid waste (e.g., bandages, gloves) from regular waste in a red plastic bag sitting on absorbent material (corn cobs) in a lidded, leak-proof outer container with a biohazard symbol on it. Ensure the location is convenient to your workbench. Incinerate it. Add non-corrosive disinfectant to a plastic carboy and pour concentrated liquid waste into it before disposal down a sanitary sewer system. Sanitize and autoclave contaminated instruments before reuse, or discard them in sharps containers before sealing, autoclaving and sanitary landfill. Place heavy waste (specimens, corpses, animal bedding) in burst-proof, rigid containers with sturdy handles before incineration. Before changing a HEPA filter in a safety cabinet, seal it with plastic sheets and tape, and steam the filter with paraformaldehyde flakes. Wear personal protective equipment (PPE) and wash your hands frequently when handling lab waste. Follow your Safety Officer's instructions for mixed waste (radiation, infectivity, and chemical contaminants).

### Hazardous chemicals

Hazardous chemicals are carcinogens (cancer-causing), corrosives (burning), irritants, mutagen (damages DNA), sensitizers, teratogens (cause fetal damage) or toxins of the bone marrow, eyes, kidney, liver, lungs, mucous membranes, nerves, and skin. They must be statistically proven in at least one properly conducted scientific study to cause significant adverse health effects in laboratory employees to be classified as hazardous. The U.S. *Department of Labor's Occupational Safety & Health Administration* requires the employer to write a *Chemical Hygiene Plan* and update it monthly for safety. It lists the lab equipment that must be used, specific procedures that must be followed, Personal Protective Equipment (PPE) that must be worn, and general safe work practices that must be observed for protection from hazardous chemicals. A *Chemical Hygiene Officer* is an employee with special training or experience the employer chooses to design and maintain the Chemical Hygiene Plan, for protection and expert advice.

### Soiled laundry

Contaminated laundry should be bagged near where it was used. Place it in red plastic bags with an orange biohazard symbol firmly attached if the laundry is being washed off-site at a commercial laundry. If your facility adheres strictly to standard (universal) precautions and does its own laundry, it can use other bag colors, providing all staff are trained on what they mean. Wear gloves and a plastic apron, and handle contaminated laundry minimally. Do not shake, sort, or rinse out the stain where the contamination occurred. If laundry was contaminated with HIV or Hepatitis B, then it must be incinerated. If laundry was just used in an HIV or Hepatitis B area, but not visibly contaminated, then it must be decontaminated first, before laundering.

### Sharps containers

A sharps safe can be any container that is red with an orange biohazard label firmly affixed to it. It must resist punctures, have a leak-proof bottom and sides, and be placed inside another biohazard container if its outside is contaminated or punctured. A satisfactory sharps safe can at least be closed, or preferably, locked to prevent theft of used syringes by drug addicts. The safe must never be reopened or reused after it has once been closed. It must be kept upright and replaced regularly, before overflowing. One of the most common causes of needle stick injuries is trying to cram one more sharp into an overflowing safe. Sharp safes must be easily accessible, and located where one would expect to find sharps, such as a laundry, operating room, examination room, neonatal intensive care unit, medication cabinet, or bleeding station. They must be autoclaved before final disposal in sanitary landfill.

### Safety training requirements

Your employer must keep for three years a record of the date you received safety training, a summary of what was covered in the session, the name and credentials of your teacher, and a class list of student names and job titles. You are entitled to free Hepatitis B vaccine within 10 days after you start work, given at a reasonable time and place, free HIV and HBV blood screening at an accredited lab, and free HBV booster shots. Your employer must record your needle stick injury in the Sharps Injury Log, including the device brand involved, the department, incident details, and identification of the source patient. You are entitled to a free medical exam, disclosure of the source's HIV and HBV status, prophylaxis, counseling, a follow-up of subsequent illness you report, and a written report in 15 days.

### Cleaning schedule

The employer is responsible for keeping the workplace sanitary. This means writing a schedule that states when and how each lab surface will be cleaned. The decontamination method depends on the type of surface material, where it is located in the facility, the type of spills, and the procedures underway in the area. Whenever any surface is in contact with blood or other material suspected to be infectious, it needs to be cleaned immediately. If protective equipment coverings are contaminated, they need to be replaced by shift's end. Garbage cans, pails, and reusable bins must be cleaned regularly and as soon as possible after they are contaminated. A brush, dustpan, tongs, forceps, gloves, disinfectant, sharps container, and paper towels need to be handy to clear away broken glass immediately. Lab coats, plastic aprons, or isolation gowns are worn for cleaning – never street clothes or unprotected uniform. If there is any possibility for splashes by body fluids, goggles and a mask, or a face shield, a cap, and shoe covers should be worn.

### Extra precautions

The extra precautions need when the laboratory is involved in HIV or HBV research or vaccine production (not just diagnostic testing):

- Allow authorized persons only into the lab.
- Post policies and procedures outlining lab dangers and entry requirements, and update them annually.
- Observe strict entry and exit procedures for animal rooms and work stations.

- Autoclave or incinerate all waste.
- Close all doors while HIV or HBV is handled in the lab, or infected animals are present.
- Post biohazard signs on all doors.
- Place contaminated material in a leakproof, puncture-proof, labeled or color-coded biohazard container; close it before removal from the workstation.
- Work only in annually certified biological safety cabinets; do not perform open bench work.
- Wear PPE at all times; remove it before leaving the area; autoclave it before laundering and reuse.
- Know how to use the eyewash station.
- Protect vacuum lines with disinfectant traps and HEPA filters.
- Use hypodermic syringes only on diaphragm bottles and lab animals, and locked needle syringes for other uses; do not bend, shear or recap needles.
- Trained staff cleans spills immediately.

### Preventing infection

The following terms regarding the prevention of infection:

- *Asepsis* prevents infection during surgical procedures by reducing pathogens. It requires sterile instruments and sterile gloves, and a strong disinfectant that can kill both gram-positive and gram-negative bacteria and their resistant spores, like 70% povidone-iodine.
- *Antisepsis* is reducing the flora and transient microorganisms on the skin for minor procedures like venipuncture. Clean gloves are worn. It requires a short-acting antiseptic like 70% isopropyl alcohol that can denature proteins.
- *Strict isolation* segregates infectious patients to one room, and visitors are restricted. Modified isolation attempts to limit infection with protective techniques, like donning gloves, gowns, and masks when handling the patient's body fluids. Reverse isolation protects a patient from others in a clean room, as after kidney transplant.
- *Standard or universal precautions* means healthcare workers control the spread of disease by assuming every patient's samples are infectious, and following the U.S. Occupational Safety and Health Administration (OSHA) standards for proper hand washing, wearing gloves, bagging specimens in biohazard bags, and disposing of needles and lancets in a sharps safe.

### **Workplace injuries for phlebotomists**

About 43% of phlebotomists suffer annual needle stick injuries from: Improper needle withdrawal; struggling patients; transferring blood from one container to another; carrying needles in a lab coat pocket; recapping a needle after use; used needles left in a patient's bedclothes by another caregiver; trying to force a needle into an overflowing sharps safe; emptying a sharp safe to reuse it instead of sealing it for disposal; handling garbage bags containing carelessly discarded sharps. The risks are for accidentally injecting toxic material or occupational blood-borne infection. There is a 30% chance the phlebotomist

will develop Hepatitis B following a needle stick injury; a 10% chance of Hepatitis C, and a 0.3% chance of HIV infection. The phlebotomist may rarely get other bacterial, viral, or fungal infections, such as: Brucellosis; cutaneous gonorrhea; diphtheria; herpes; malaria; mycoplasma caviae; Rocky Mountain spotted fever; staphylococcus; streptococcus; syphilis; toxoplasmosis; tuberculosis.

### **Needle stick first aid**

Phlebotomists must follow the OSHA bloodborne pathogens standards and keep all immunizations up-to-date. Most occupational health nurses administer free Hepatitis B vaccine as prophylaxis for at-risk staff. When a needle stick injury occurs:

- Let it bleed freely and wash the wound immediately, ideally with povidone-iodine.
- Report it within 24 hours to your employer.
- Fill out any workers' compensation forms and keep copies.
- If you are breastfeeding, stop until your doctor advises you otherwise.
- Request prophylactic Hepatitis B immune globulin (HBIG) to boost your antibodies. This may help even if your immunization did not include Hepatitis B.
- If you know which patient contacted the particular sharp that cut you, you may request disclosure of his/her disease status.
- If the patient is a known carrier of HIV, you may consider taking AZT and getting antibody tests at baseline, three months, and six months after exposure. There are health and insurance risks associated with this decision, so consult your doctor first.

### **Quality control**

#### ISO quality management system

The International Organization for Standardization (ISO 9001 – 2000) set the current quality management system used by medical laboratories, car manufacturers, and aviation. Subsections that apply to medical labs are 17025 (reference laboratory testing and calibration) and 15189 (clinical laboratory quality and competence). Quality management makes it easy to do the right thing and hard to do the wrong thing. A quality management system means all staff are trained and follow safe, accurate processes consistently. Problems arise from incorrect processes, not people. Quality management involves asking yourself if the service you provide is appropriate, efficient, effective, and high-quality. ISO standards require your lab manager to have a policy and procedure manual that defines all your activities, monitors and measures their effectiveness, and encourages continuous improvement. Your lab processes must match exactly the documented procedures. You must know exactly how to do a job, where to find information, who is responsible, and how your job is linked to others'. Your goal is to reduce 98,000 annual U.S. deaths from medical errors.

### Correcting faulty lab processes

When a faulty lab process is found as part of an audit:

- Bring it to the attention of the quality manager.
- Gather proof (requisitions, results, incident reports, quality control records)
- Map the process now used.
- Review the OSHA and CLIA requirements pertaining to the problem.
- Find gaps in the process.
- Find gaps in the documentation.

The quality manager will prioritize the problem, plan and document the new process. Verify that the new process works, determine if it affects any other processes, and give the quality manager feedback. The quality manager surveys co-workers and patients, trains the staff, and sets a time to implement the new process. Help the quality manager measure how effective the new process is, and to modify it if required. The quality manager will update the manual.

### ASCLS quality controls

ASCLS suggests:

- U.S. Department of Health and Human Services to collect standardized reports of all adverse and sentinel events and allow the public access to them
- Error management education for all staff
- Review of medical errors during accreditation, certification, or licensing
- Raised standards for all healthcare professionals
- Patient identification system to be used from the patient's first visit
- Specimen bar coding for identification
- Raised awareness of medical errors (iatrogenic disease)
- Tools to reduce errors using evidence-based information and personal anecdotes
- Laboratories "provide adequate space and instrumentation, sufficient supplies and support staff, ergonomically sound design, and personal protective equipment"
- Each lab appoints a Patient Safety Manager

### **OSHA**

The U.S. Department of Labor's Occupational Health and Safety Administration has a Web site at <http://www.osha.gov> that provides important updates you are required to adopt as part of your standards of practice. Your Safety Officer must check the site regularly, and will notify you of any pertinent changes, and arrange for your training at your employer's expense. You need to check the site:

- Whenever you need a refresher about established safe practice, like bloodborne pathogens.
- Whenever a serious new threat develops, like an influenza pandemic.
- When equipment or supplies change in your laboratory, to make sure your current best practice for infection control and hazardous chemicals covers the change.



- When contemplating a grievance against your employer for allowing unsafe practices, to make sure you know exactly what the correct standards are before registering a complaint.

### *Hygiene standards*

Your employer measures, cleans, repairs, and disposes of your personal protective equipment at company expense. You must remove PPE before leaving the laboratory. OSHA forbids you to apply make-up, lip balm, or other cosmetics (e.g., nail polish, sun screen) in your laboratory workplace because they could become contaminated. OSHA does not allow you to handle your contact lenses in the laboratory. You are not permitted to put beverages or food on lab countertops, shelves, or in supply cabinets, or specimen or reagent refrigerators and freezers. You are not allowed to bring food and drink into the lab or consume them there, or into the reception area where patients drop off specimens. If you are performing tolerance tests that require you to administer drinks (e.g., glucose, lactose, and lactulose), then you must keep them in a separate refrigerator, away from specimens. You must not smoke in the laboratory, as it could not only affect results and annoy the other staff members, but could infect you.

### *Hand washing technique*

To wash your hands correctly, first remove your jewelry. Use clean, dry paper towels to turn on the taps. Use warm water to avoid skin damage. Wet your hands and apply disinfectant soap (povidone scrub or bar soap rinsed and stored in a drainer). Count to 30 while scrubbing the backs and palms of your hands with the lather, and interlace your fingers while rubbing them together. Brush gently under your nails. Note any cuts, rashes, broken or long nails that need treatment before resuming work. Rinse well and dry your hands with paper towels, not a blow dryer. Use clean paper towel to turn off the taps and to open the exit door. If there is no sink nearby, use 70% to 80% alcohol cleanser (Cutan, Florafree, Manorapid, Purell) for 15 seconds, followed by disposable antiseptic towelettes (benzalkonium chloride). Change gloves frequently by turning them inside out from the wrists. Wash your hands as soon as possible.

### *Gloving before blood collection*

OSHA requires phlebotomists to glove before every venipuncture as part of standard (universal) precautions for bloodborne pathogens. Only if your employer runs a volunteer blood donation center does OSHA allow for ungloved venipunctures, providing:

- The phlebotomist is experienced. Trainee phlebotomists must glove.
- The phlebotomist agrees not to glove. The employer must provide gloves if an employee requests them, and if the employee is allergic to latex or powdered gloves, the employer must provide hypoallergenic or unpowdered gloves, liners, or barrier cream.
- The phlebotomist's skin is intact, i.e., it does not have any visible burns, rashes, cuts, or scratches.
- The patient is co-operative. If the patient is likely to struggle or have a reaction, then the phlebotomist must glove.

Your employer cannot discourage you from using gloves for blood collection. Your employer must reevaluate the no-glove policy periodically to see if it continues to be a low-risk option. In circumstances where there will almost always be contact with blood, like infant heel pricks, gloves are required.

#### *CBRN event versus OSHA emergency*

A CBRN event is bioterrorism or an industrial accident that releases chemicals, biologicals, radiation, or nuclear waste and requires the assistance of local officials (police, fire, ambulance, Public Health, and the IMS Team) and your help with decontamination. An *OSHA emergency* is whenever any physical hazard is released into your workplace in an uncontrolled way. This could be because its container breaks or leaks, or if control, storage, or testing equipment malfunctions (e.g., filters, refrigerator, Coulter Counter). Physical hazards are combustible liquids, compressed gases, explosives, flammables, pyrophoric chemicals that ignite above 130°F, chemicals or metals that react in air or water, organic peroxides and oxidizers.

#### *Fume hoods, HEPA filters and OSHA's formaldehyde gas regulations*

A fume hood, or BSC, is a workplace for toxic chemicals, tissue grinding, or infectious body fluids that is shut in on five sides, and partially enclosed on the sixth side to allow the technician access with covered hands and arms. The fume hood draws air from the lab and prevents as much aerosol as possible from returning to the lab. Fume hoods are equipped with High Efficiency Particulate Absorbing (HEPA) air filters that absorb 99.97% of particles 0.3 microns or larger from the air, including allergens, bacteria, dander, dirt, dust, fungi, pollen, and some viruses, and have ultraviolet light shining on them to speed killing by dehydration. N100 respirator masks are the equivalent of a HEPA filter on your face. You must wear a respirator if you will be exposed to more than 0.5 parts per million of formaldehyde in the air during an eight-hour shift. Your plant operations manager ensures lab air is exchanged 6 to 12 times every hour wherever carcinogenic formaldehyde is used.

#### HIPAA

##### *Title II of HIPAA*

The intent of Administration Simplification (Title II) of HIPAA was to reduce healthcare costs by stopping healthcare fraud, ensure patients have privacy, and secure patients' personal information. The Department of Health and Human Services standardized the way healthcare providers, billing services, and community health information systems perform electronic transactions, the type of information they can keep, and how they can use it. Each employer, insurer, and healthcare provider now has a national identifier. The Privacy Rule for Protected Health Information outlines what your provider, insurer, or billing company can disclose from your medical record. This includes how you pay, payment history, provider's name, or what your health status is. The disclosure must be minimal, has to be documented for a legitimate purpose, and the patient must be told about it within 30 days. Every patient has the right to have errors corrected on his/her file, and to complain to the Privacy Officer and the Department of Health and Human Services for Civil Rights.

### Security Rule

HIPAA's Security Rule requires that you have Administrative, Physical and Technical safeguards in place at your facility to protect patients' rights. That means you must have clear policies and procedures in place (P&P) that state:

- A management person oversees them
- The workers agree to follow them as a condition of employment
- The classes of workers who have access to confidential information
- Security controls in detail
- Routine audits will be performed
- Adverse event audits will be performed
- Emergency back-up plans in case of a breach, with detailed instructions for follow-up

You must monitor data access and protect it by allowing only properly trained and authorized persons to access it. You must control access to your computer system and protect it from intrusions, like viruses and spyware.

### CMS and CLIA

Ten regional offices of the Centers for Medicare and Medicaid (CMS) throughout the U.S. provide health insurance for about 74 million vulnerable Americans through fee-for-service. The Social Security Act of 1965 governs Medicare, Medicaid, and Child Health. The CMS controls American labs through the Clinical Laboratory Improvements Amendment (CLIA). The only exception to this control is private research labs, which are exempt.

- *Medicare A* is no-cost insurance for Social Security recipients over 65 years old, and patients with renal failure (dialysis patients) in these facilities: Critical access hospitals; home care; in-patient hospitals; hospices; skilled nursing facilities.
- *Medicare B* insurance covers out-patients for \$88.50 per month for: Blood transfusion; diagnostic tests like glaucoma, Pap smears, mammograms, and prostate exams; doctors' office visits; durable assistive devices, like beds, oxygen, walkers, wheelchairs; laboratory tests; one physical exam in the first 6 months; out-patient clinics for mental health, occupational therapy, and physiotherapy; out-patient surgery; vaccines, like Hepatitis B and Pneumococcus.

### Peer assessment

JCAHO peer assessment visit at accreditation renewal time and required duties are following. A peer assessment is conducted to make sure the lab:

- Conforms to ISO and CLIA standards, state and federal legislation.
- Hires competent people who follow quality requirements.
- Conforms to the scope of tests on its license.
- Stays current and meets any special challenges.
- Has a quality manual that explains how to collect, store, ship, and test specimens.
- Continuously looks for problems and corrects them quickly.
- Gets accredited and remains open for business.

As part of the accreditation review, you might be asked to:

- Fill out questionnaires for the assessors.
- Help prepare an agenda.
- Book conference rooms and audio/visual equipment.
- Arrange lunches.
- Book briefing and debriefing sessions.
- Get visitor passes and lab coats for the assessors.
- Arrange for the assessors to interview doctors and nurses associated with your lab.
- Review OSHA standards and your quality manual.
- Produce conformance evidence (log sheets, medical records, inventory records).
- Help find solutions to non-conformance issues in 90 days.

### **Risk factors yielding errors**

The risk factors your patients could have that would predispose their test results to errors or to the patient having complications after testing:

- *Allergies:* Patients may forget to tell you about allergies to latex gloves, tape adhesive, medication, or contrast dyes.
- *Age:* Very young and very old patients are fragile and susceptible. They do not control internal temperatures well. Their normal values may be different than young adults'.
- *Central Nervous System disorders:* Unsteady gait, convulsions, weak or spastic muscles, and tiredness all contribute to falls. Patients with canes, walkers and other assistive devices can be slow-moving.
- *Drug use:* Patients who mix over-the-counter (OTC) medicines or herbs with prescription drugs, abuse alcohol or street drugs, or who take sedatives or strong analgesics that cause drowsiness are at-risk.
- *Immunosuppression:* Patients with AIDS, cancer patients receiving chemotherapy or radiation, chronically ill patients, and organ recipients who are taking Cyclosporin to prevent organ rejection all have weakened immune systems and are infection-prone.
- *Psychological problems:* Aggressive patients, those who take hallucinogens or phencyclidine, have uncontrolled pain, are restrained, or have psychiatric problems are a challenge.
- *Sensory impairments:* Blindness, deafness, imbalance, or paresthesia can make tests difficult.

### **Disease incidence**

Disease incidence measures how prevalent a disease is among a given population in a specific place, over a specific time. Incidence predicts how probable it is a patient will develop a disease, and its etiology (likely cause). Predicted values estimate how likely a test result is to be right or wrong, given certain variables, like the patient's age, occupation, race, income, how long the symptoms have lasted, and if there is fever. Positive predictive

value is the likelihood that a patient with a positive test result really has the condition. Negative predictive value is the likelihood that a patient with a negative test really does not have the condition.

### **Sensitivity and specificity**

Sensitivity is also known as the recall rate. It measures how many times a test produces true-positive results, indicating patients probably have a disease, compared to the gold standard test for that particular illness. Sensitivity is important for early detection of disease, or to stop an epidemic. To calculate the percentage of sensitivity, divide the number of patients who really have the disease and test positive by the total patients tested who have the disease (including those who tested false-negative), and multiply by 100. Specificity measures how many times a test produces true-negative results, indicating patients probably do not have a disease, compared to the gold standard test for that particular illness. Specificity is important when treatment is toxic and could harm a patient, like chemotherapy. To calculate the percentage of specificity, divide the number of patients who really do not have the disease and test negative by the total patients tested who do not have the disease (including those who tested false-positive), and multiply by 100.

### **Retesting**

When a sample result is abnormal, it is better to alert the doctor and let him/her decide whether to call the patient back for a repeat test. The sample may, in fact, be a “panic value” (critical result) where the patient needs to be called back STAT for resampling. However, check the margin of error. For example, the lab test is accurate to the 95<sup>th</sup> percentile, and the first result is abnormal. This patient happens to be among the 5 in 100 who tests abnormally even when healthy. The technologist repeats the test on the same specimen. You can calculate the probability that the second result will also be abnormal with this formula:

$$\begin{aligned}0.95 \times 0.95 &= 90.25\% \\100\% - 90.25\% &= 9.75\%\end{aligned}$$

The healthy patient now has an even greater chance of abnormal test results second time around: 9.75% probability of a second mistake, instead of 5% on the first test. Each time the specimen is retested increases the chance that the result will be falsely abnormal.

### **Mean, median, and standard deviation**

Mean is the value obtained by dividing the sum of a set of quantities by the number of quantities in the set. It is also called average. Median is the midpoint of the range numbers that are arranged in order of value. Standard Deviation, in statistics, is the average amount a number varies from the average number in a series of numbers.

## Mode and coefficient of variation

Mode is the most frequent score. For example, your kidney transplant patient has hemoglobin levels taken for a week following the surgery.

Hemoglobin in g/dL
15.0
12.0
12.0
12.0
10.0
8.0
7.0

The most frequent hemoglobin test result is 12.0 g/dL, so 12.0 is the mode. Coefficient of variation is the standard deviation divided by the mean, multiplied by 100. Its short form is %CV. It tells you how precise a test is. The lower the percentage, the more likely it is that there was no variation in the way a test was performed from sample to sample. Ideally, there should be no more than 4% coefficient of variation. Remember that your lab can have a low coefficient of variation, but can still be making the same testing mistakes over and over again because of poor quality control. Coefficient of variation also allows you to compare the differences (inequalities) between two populations.

## Managing test results

The steps for a phlebotomist to effectively manage test results and avoid malpractice charges:

1. Log all tests collected and send-out lab name
2. Track send-out tests until all results are received back
3. Call the reference lab to find out reasons for delays
4. Call back patients for repeat testing if the sample was lost, broken, insufficient, untestable, or gave equivocal results
5. Flag abnormal results
6. Ensure the doctor reviews all results
7. Inform the patient of normal results in a timely manner, if authorized by the doctor
8. Give numerical results to well-regulated patients with chronic conditions, where the doctor has discussed disease management and the meaning of the results with the patient beforehand (e.g., diabetics, dialysis, and anticoagulant patients)
9. Document that the patient was informed of results by a staff member
10. Ensure the doctor's recommended follow-up occurred (e.g., inform patient of prescription order)
11. Book a return appointment for the patient to meet with the doctor to discuss abnormal results
12. Mail a copy of the results to the patient

## Confidentiality practices

When training a temporary replacement, the following need to be communicated regarding confidentiality practices:

- Never carry on a conversation with an unidentified caller, or directly tell a caller if the patient is in the office. Reporters, lawyers, and domestic violence perpetrators use this tactic to track people. Always get the name of the caller. Say you will look to see if the patient is there. Ask the patient if he/she wants to take the call.
- Turn over confidential papers and schedules and turn off your computer screen when you leave your desk. Angle screens and papers so that visitors cannot read them while you work.
- Use low background music to help blur conversations for eavesdroppers.
- When talking about a patient in a crowded area, try to use only one name, e.g., Mrs. Bartleson or Jane, not both.
- Get a signed consent to disclosure form before releasing confidential information.
- You're a Business Associate under HIPAA, so you must protect patient information and report unintentional disclosure. You can be dismissed and charged if you fail to do so.

Three instances when law overrides the patient's right to confidentiality are:

- The doctor must report some infectious diseases to Public Health to prevent an epidemic. These include sexually transmitted diseases and tuberculosis.
- Caregivers' have the right to know the patient's diagnosis if it poses a risk to them (infection, likely to assault).
- Suspicion of child abuse: Since 1996, all states require doctors, nurses, chiropractors, psychologists, social workers, law officers, daycare staff, clergy, and teachers to alert local authorities if they suspect child abuse or neglect under the Child Abuse Prevention and Treatment Act (CAPTA). Abuse and neglect occur when a person who is responsible for a child under 18 commits or omits an act that may cause death, serious emotional or physical harm, sexual abuse or exploitation. Each state has an abuse hotline. Many states require anybody who has reasonable cause, in good faith, to believe a child or elder is being abused to report it or face civil liability.

## Spectrophotometry

A spectrophotometer (Spec-20) compares the intensity of light entering a sample and exiting from it (percent transmittance) to find the concentration of the sample. Visible spectrum light ranges from 440 nm to 700 nm. Different substances absorb different light wavelengths. Beer's law states absorbance is proportional to the concentration of a solution. A completely transparent sample has 100% transmittance. A completely opaque sample has 0% transmittance. Beer's law only applies if absorbance is between 0.1 and 1.0.

Grind your sample with 25 mL of 6M HCl in a 250 mL beaker. Cover and boil gently in a fume hood for 15 minutes. If volume falls below 15 mL, add distilled water up to 25 mL.

Let cool. Filter out any solids into a 100 mL flask. Pipette 1 mL into a 50 mL flask. Add hydroxylamine hydrochloride, sodium acetate, 1,10-phenanthroline and distilled water to the line and place in the spectrometer on a clean cuvette. If you need to correct the solution because the sample contained dye, omit the 1,10-phenanthroline.

### How to use a spectrophotometer

The Biomate 3 is typical; all spectrophotometers work similarly. Use quartz cuvettes when measuring DNA and stock solutions. Use disposable plastic cuvettes when measuring bacteria. Wipe the windows on the quartz cuvettes with 95% ethanol and a Kimwipe. Do not use Kleenex or acetone, as they will damage the \$700 quartz cuvettes. Measure your stock solutions first. Choose *Smart Start*. Choose *Oligo*. Refer to your oligo sheet and enter the conversion factor in mcg/OD. Place 995 mcL of MQ water on a 1 mL cuvette. Load it into the cuvette holder. Choose *Run Test*, then *Measure Blank*. Add a 5 mcL sample or media to the cuvette. Mix by inverting and replace it in the cuvette holder. Choose *Measure Sample* or *Cell Growth*. If the oligo is diluted, use a microcuvette. Add 90 mcL, choose *Measure Blank*, add 10 mcL diluted solution, and vortex. A260 should be above 0.1. Choose *Esc*. Save test results. Wash out the cuvettes with MQ water and 95% ethanol and blow dry.

## **Microscopes**

### Main parts of the microscope and why they are needed:

- *Aperture*: Hole in the middle of the stage. Light shines through it onto the slide.
- *Arm*: Joins the eyepiece, stage and base. Used to carry the microscope securely.
- *Base*: Weighted stand below the stage to keep the microscope upright.
- *Knobs*: Coarse adjustment knob moves objectives up and down. Fine adjustment knob clarifies the view. Stage knobs move the slide around to change the field in view.
- *Lighting*: The base illuminator dome light is below the stage. Behind the base illuminator is an on/off switch. Above the base illuminator, on the bottom of the stage, is a light adjustment lever for low, medium and high lighting.
- *Objectives*: Low power, high power, and oil immersion objectives hang down from the revolving nosepiece.
- *Ocular*: Eyepiece(s) at the top of the microscope (monocular or binocular).
- *Revolving Nosepiece*: Under the ocular, above the stage, the revolving nosepiece holds the objectives steady.
- *Slide Clip*: Holds the glass slide in position on the stage.
- *Stage*: Flat surface under the objectives on which the slide rests.

### Cleaning and storing a microscope

There are five glass areas on the microscope made of soft, optically ground glass. Kleenex and paper towels are too rough to safely clean this expensive type of glass because they will scratch it. Lightly dip lens paper in xylene or another oil solvent. Clean the ocular first, then the low, high, and oil immersion objectives, and lastly the stage aperture. Wipe the entire stage. Polish the glass and stage with a fresh, clean, dry piece of lens paper. Rotate



the low power objective clockwise into place with the revolving nosepiece, and raise it to the highest position with the coarse adjustment knob to avoid scratching the lens. Wrap the power cord around the base. Drape the microscope with a plastic cover or put it in a microscope box. Keep it away from temperature extremes and moisture. Carry it only by the arm and avoid bumps or falls.

### Calculating magnification

The ocular(s) are usually marked 10x magnification. (Small office models are sometimes 5x magnification.) The low power objective is marked 10x magnification. If you use a 10x ocular with a low power objective, then  $10 \times 10 = 100$ , so you are looking at an object at 100 times its actual size. The field you see is actually around the size of three pin heads. The high power objective is marked 43x or 44x, depending on the model. If you use the 10x ocular and the high power objective, then  $10 \times 43 = 430$ , and  $10 \times 44 = 440$ , so you are viewing an object at 430 or 440 times its actual size.

The oil immersion objective requires special oil on the slide in order to focus properly. It is marked 100x magnification. If you use a 10x ocular and the oil immersion objective, then  $10 \times 100 = 1,000$ , so you are viewing an object at 1,000 times its actual size. The field is pinpoint size.

### When a smear cannot be identified

If you find something on a smear you cannot read or that is very significant, use the vernier scales on the right and bottom sides of the stage to mark your place. Center the item that you cannot identify in the middle of the field by using the stage knobs. One knob moves the slide horizontally and one moves it closer or farther from your eyes. Carefully note the readings on the two vernier scales, e.g., Bottom 24, Side 40. Then you can safely resume reading the slide, and still return to the unusual item easily. Avoid jiggling the slide or allowing it to dry out, because movement or evaporation may change the location of the item. Inform the charge technologist or doctor that you need assistance to identify the item, or that you have found a significant structure and want it confirmed.

### Focusing a slide clearly

Use the correct stain, and a cover slip on top to prevent evaporation if it is a wet mount. Place the slide on the stage. Clip it down to secure it. Plug the microscope into an electrical outlet. Turn on the base illuminator with the light switch. Turn the light to low by moving the lever to the far right. Make sure the low power 10x objective is locked into position with the silver knobs on the revolving nosepiece. Turn the coarse adjustment knob away from you to lower the objective towards the slide. Watch from the side so you do not grind the soft lens into the slide and damage both – keep the objective just above the slide. Rotate the fine adjustment knob away from you. Look into the ocular with both eyes to avoid eye strain. Turn the coarse adjustment knob towards you very slowly to focus the slide. Stop when an object can be seen. Turn the fine adjustment knob towards you to bring it into sharp focus.

### Viewing fine details with an oil immersion lens

Center the object you want in the middle of the field by using the stage knobs. The oil immersion lens is the longest, so use the coarse adjustment knob to raise the lower power objective and allow enough room for the longer lens. Turn the objectives clockwise to oil immersion. Place a drop of immersion oil on the slide below the lens. Use the coarse adjustment knob to lower the long objective near the oil drop. Watch from the side as you turn the fine adjustment knob away from you to slowly lower the lens into the oil. Look through the ocular and focus the object with the fine adjustment knob. Move the light adjustment knob left to brighten the field. Once the object is focused, do not move the lens in and out of the oil, but keep them in contact as you move through the fields. Clean the immersion oil from the lens with xylene and lens paper before using the microscope for another specimen, or storing it.

### **Operating a centrifuge**

If you do not balance your centrifuge before using it, the blood may hemolyze because of motor vibrations. An unbalanced centrifuge “walks” across the workbench and may fall. Glass tubes may break in an unbalanced centrifuge, requiring lengthy cleanup and disinfection. Place sealed tubes containing approximately equal volumes opposite each other in the centrifuge cups. If you have only two tubes with very unequal amounts of blood (e.g., 3 mL and 10 mL), or an unequal number of tubes, then match them across from counterweight tubes filled with water of the corresponding volume. Never open a centrifuge while it is still spinning, as broken glass and dangerous aerosols may fly out. Wait until the centrifuge stops completely before opening it. Do not jiggle the tube as you remove it, because the solids will resuspend in the liquid. Each day, wipe down the outside with a damp cloth. Remove broken glass with forceps. Clean spills with alcohol on a cloth and air dry the parts.

### **Lab refrigerators**

Lab refrigerators are used to store highly volatile, flammable liquids. They must have no sources of ignition that would be in a domestic fridge, such as heater strips, inner thermostats, light switches, and compressors and circuits at the base where flammables can accumulate. They must be explosion-proof, have self-closing doors, thresholds, friction latches, magnetic door gaskets, and a compressor and circuits at the top. They must be manually defrosted, and have continuous recording thermometers, set between 0°C to +6°. Blood bank requires +4°C. Class IA, IB, and IC are flammables. Class II, IIIA, and IIIB are combustibles. No more than 120 gallons of Class I, II, and IIIA liquids can be stored in a lab fridge, and of those, no more than 60 gallons may be Class I and II. Do not locate more than three storage cabinets in one fire area. No more than 50% of the flammables can be stored for teaching. Use DOT approved glass, metal or polyethylene containers no larger than 1.1 gallons (4 liters).

## Biochemistry laboratory machines

Johnson and Johnson's Vitros DT60 II is good for point-of-care (bedside or clinic) routine chemistry testing or as a small backup machine in case the main analyzer fails. Randox Daytona is a benchtop system for large labs because it can test up to 40 specimens at a time and it automatically barcodes specimens and refrigerates the reagents. The Roche/Hitachi Analyzer performs 26 routine chemistry tests, like electrolytes and proteins, on CSF, plasma, serum, and urine, and is good for small labs with low patient volume. The Roche Cobas Integra tests 36 routine tests, like protein, and common drug levels like lithium. Cobas is good for labs with limited space and staffing, because the reagents inventory is automated and it is very sparing when it uses reagents. The Abbott ARCHITECT Analyzer is used for chemiluminescent immunoassay (CMIA), to perform tests like AFP and Beta HCG. The Abbott AxSYM is used performs microparticle enzyme immunoassay (MEIA) for CEA.

## Aliquot

Aliquot is dividing a chemistry solution into equal parts. It is used for very expensive reagents, which are measured out into smaller solutions as needed, instead of using the whole bottle. Aliquot is also used to make adjustments to blood samples or drugs that are below scale. For example, a chemistry machine can test blood samples as small as 15 cc, but you have many tests ordered and not enough blood in separate tubes. You can proportionally dilute the sample to make it testable. For example, 3, and 5 are both divisors of 15, so you can make aliquots in those equal proportions. Beckman Coulter makes an aliquotter machine that moves serum from one collection tube into aliquot tubes. Most aliquots are automated in today's laboratories.

## Blood collection tubes

The types of blood collection tubes by color of stopper, additive, and give examples of tests commonly performed on each:

Color	Additive	Tests
Black	Sodium citrate	ESR
Brown	Heparin	Lead
Gold or marbled	SST gel separator	Routine Chemistry
Green	Heparin	Electrolytes, gases, and hormones
Grey	Potassium oxalate and sodium fluoride	Glucose
Lavender	EDTA	Complete blood count (CBC)
Light blue	Sodium citrate	Prothrombin time (PT) and (PTT)
Navy	None, free of all trace elements	Toxicology, heavy metals
Pink	None	Public Health and Blood Bank
Red	None	Blood Bank, Routine Chemistry, Serology
Yellow	(SPS)	Blood cultures, Blood Bank tests

## Microtainer pediatric tube

After you puncture the child's heel with a short point lancet, draw blood into the Microtainer tube by capillary action and GENTLE squeezing in this order:

Color	Additive	Order of Draw	# of Inversions
Lavender	EDTA	First	Mix 20 times
Dark Green	Lithium Heparin	Second	Mix 10 times
Mint Green	PST	Third	Mix 10 times
Gold and Amber	SST	Fourth	Do not mix
Red	None	Last	Do not mix

Newborns have thin skin. Using a long point lancet could nick the calcaneus, and cause osteomyelitis so that the baby has to have the injured foot amputated. Wiping the puncture with alcohol to encourage bleeding when the wound has already clotted will dilute the specimen. Vigorous squeezing can cause interstitial fluid to leak into the specimen and dilute it, or hemolysis of red blood cells, giving a false result.

## Vacutainer, butterfly, lancets and syringes

When it is appropriate to use an evacuated blood tube (Vacutainer) and cannula, a butterfly needle (Portex Saf-T Wing), lancet, and syringe:

- Use a 20, 21, or 22 gauge *Vacutainer* system when a quick, high-quality blood sample is needed from a conscious, adult patient or older, compliant child with adult supervision. It is not suitable for very young children, noncompliant children, patients with severe bleeding disorders, thickened skin conditions like scleroderma, or microbiology specimens.
- Winged or *butterfly* 23g needles are for difficult collections, like small veins, jugular veins, small children, bleeding disorders, and frail elderly patients. It is the best tool for collecting blood cultures because it keeps the media broth from flowing back into the patient's vein. Blood donations use 19g or 20g needles.
- *Long point* lancets are for adult finger and ear lobe microcapillary collections, like diabetic glucose checks. *Short point* lancets are for babies' microcapillary collections.
- *Syringes* can be used for bone marrow aspirations, and when a butterfly is unavailable. Syringes are for some hormone and tissue typing tests where large amounts of whole blood are transferred to plastic or glass beaded tubes.

## Venipuncture collection

### The equipment to be assembled before a routine adult venipuncture collection:

- Completed requisition with a physician's signature and billing information
- Soap, water, and towels for hand washing
- Sufficient evacuated blood tubes with the right color stopper
- One 21g or 22g cannula × 1.5" length
- Plastic Vacutainer holder with a luer-lock hub
- Isopropyl alcohol or povidone-iodine swab
- Clean, dry cotton balls
- Band-aid or Micropore tape, if the patient is allergic to adhesive
- Tourniquet (rubber Penrose catheter or blood pressure cuff )
- Kidney basin or tray to hold the specimen during collection
- Latex or vinyl gloves
- Pen with indelible ink
- Plastic biohazard bag with outer pouch for the requisition
- Well-buttoned lab coat to protect your clothing
- Reclining chair or bed to support the patient
- Certain tests require chemical additives, ice, or a hot water bath
- Sharps disposal container
- Garbage can

### The patient identification and consent procedures needed before attempting venipuncture:

1. If the patient requires an interpreter, get one.
2. Tell the patient which doctor requested the blood sample.
3. In general terms what you are going to do.
4. Check the patient's arm band or health card to confirm identity.
5. Verify the correct spelling of the patient's name and date of birth.
6. If there is any discrepancy between the patient's written identification and your requisition, STOP. Get a doctor or nurse who knows the patient to confirm the identity, and note this on the requisition.
7. A conscious, adult patient has the right to refuse treatment. If you proceed with collection after the patient has refused it, you can be charged with battery. Mark "PATIENT REFUSED" with your initials, the date and time on the requisition, return it to the patient's chart, and inform the charge nurse at once. If the patient is unconscious, the law considers you have been given implied consent.

### The preparation steps for venipuncture:

1. Check the expiration dates on all tubes, cannulae and swabs. Get fresh products if any have expired.
2. Wash, dry, and glove your hands.
3. Place the equipment in the kidney basin on an easily accessible table, not on the bed where they can break if the patient rolls over.
4. Select the patient's arm that has no intravenous fluid drip or injury.
5. Tie the tourniquet around the patient's bicep, or inflate a blood pressure cuff to 20 mm/Hg.
6. Ask the patient to clench a fist. Palpate the veins in the ante cubital fossa.
7. If the arm turns blue, or if the patient complains the tourniquet is too tight, loosen it immediately. Wait until the arm returns to a normal color before reapplying. Prolonged or repeated use of the tourniquet or blood pressure cuff can result in artificially high calcium levels in the specimen.

### Best veins to choose for venipuncture

If there is an I.V. near the only suitable collection site, inform the patient's nurse that it must be turned off for at least one hour in order for an accurate sample to be obtained. After one hour, draw blood below the transfusion site only. Note this variation on the requisition for the testing technologist. The safest vein to choose is the median cubital. If that is unsuitable, try the cephalic, but remember it tends to roll away from the cannula. The last choice is the basilic, which tends to bruise. If all these veins are unsuitable, examine the patient's hands for a superficial, large vein. The best vein will feel rubbery and will "bounce back" under the light pressure from your finger. Avoid scarred or damaged areas.

### Proper needle gauge and length:

- *Large arm veins or obese patient:* 21-gauge × 1.5inch cannula. A 21-gauge cannula is always preferable, because it has the same bore as a 20-gauge cannula, but is thin-walled. This means less hemolysis and clotting during collection, and consequently, a better sample for testing. Attach the cannula to the holder by screwing it clockwise into the hub.
- *Small arm veins or hand collection:* 22-gauge × 1 inch cannula.
- *Fragile veins or blood culture collection:* Butterfly needle with povidone-iodine swab.

### Cleaning the collection site:

- Clean the patient's arm with an isopropyl alcohol or iodine swab in an outwards spiral motion from the centre of the venipuncture site. Wait for the disinfectant to dry before puncturing the skin, to avoid unnecessary pain to the patient and contamination of the specimen. Do not touch the area you cleaned again during the procedure.
- While waiting for the disinfectant to dry, use a fresh alcohol swab to clean the stoppers of the collection tubes. Discard dusty or soiled tubes in the sharps container.

Arm position and changing tubes:

1. Position the arm on a pillow so blood flows downwards.
2. Tell the patient to remain still.
3. Retract the skin from the site, so it does not clog the bevel.
4. Uncap the cannula.
5. Rest the first tube inside the holder. Do not push it onto the hub.
6. Insert the cannula, bevel up, into the vein at a 30° angle.
7. Grasp the holder with one hand. Push the tube onto the hub with the other. Blood flows automatically into the evacuated tube when the stopper is pierced, and stops when its vacuum is depleted.
8. Detach the filled tube from the end of the cannula. Invert the tube several times to mix the anticoagulant and blood. Place the filled tube in the kidney basin.
9. Repeat Step 7 and 8 until all the tubes are filled.
10. As the last tube fills, release the tourniquet to relax the pressure, so blood does not spurt when you remove the cannula from the patient's arm.

Troubleshooting for when no blood flows into the Vacutainer tube:

- Maybe the vacuum has been lost. Replace it with a fresh tube.
- If the cannula is inserted too deeply, perhaps the tip has pierced the back wall of the vein, and is resting in the muscle. Pull out the cannula very slightly to reposition it within the lumen of the vein.
- If the skin is not tightly retracted, a loose bit could block the bevel. Without removing the cannula from the vein, rotate it so that the bevel is down.
- If the patient shifted or clenched the muscles very tightly, the cannula could bend and slow the flow. Try to reposition it very carefully.

If none of these steps work, try one more venipuncture. If unsuccessful, ask the patient if he/she will let somebody else try. Do not perform more than two venipunctures on the same patient. Get help. If your helper is unsuccessful, write "UNABLE TO OBTAIN BLOOD" and your initials on the requisition. Ask the doctor for a femoral stab.

### Venipuncture adverse reactions

Certain patients may react adversely to venipuncture. The minor change in blood pressure during collection may cause a severe epileptic to convulse. An anxious patient may faint at the sight of blood. A fasting patient may have low blood sugar (hypoglycemia) that causes fainting. This is the correct first aid for syncope:

1. STOP THE COLLECTION.
2. Call out for help or press the call bell.
3. Apply direct pressure to the puncture site.
4. Tilt the bed or chair so that the patient lies flat and blood flow to the brain is re-established.
5. Ensure the patient does not fall or sustain injury, but do not restrain a convulsing patient.
6. Give the conscious patient water or a stimulating drink to replace lost volume.
7. Note the incident on the requisition and in the patient's chart.
8. Notify the doctor and your risk manager.
9. Tell your patient to warn the technician in future collections that he/she needs to lie down.

### Preventing bruising

1. Before removing the cannula, remove the tourniquet and rest cotton over the puncture site.
2. Remove the cannula while still on the end of its holder. Do not unscrew the cannula first.
3. Apply steady pressure to the wound. It will take 4 to 6 minutes for the average patient to stop bleeding – longer if the patient is taking anticoagulant drugs like aspirin or Coumadin. Ask the alert patient to continue applying pressure after the first minute, so you can dispose of the used equipment.
4. Elevate the puncture slightly to help slow bleeding, but do not bend the arm.
5. When the bleeding has stopped, check the puncture site. If you notice a bruise (hematoma) forming, apply a pressure bandage and an ice bag over the site for 20 minutes.
6. Apply a Band-Aid or clean cotton and Micropore tape over the clotted wound, as the patient prefers. Instruct the patient to leave the dressing in place for at least an hour.



### Cleaning up safely after a venipuncture:

1. Do not recap the cannula. Dispose of used cannulae and broken tubes in the sharps container.
2. Dispose of gloves and cotton in the garbage can.
  - a. Patient's name
  - b. Health insurance number
  - c. Date and time of collection. The time is especially crucial for blood sugar and hormone tests.
  - d. Initial the tubes.
3. Label the tubes in indelible ink with:
4. Place the tubes in a biohazard bag, with the requisition in the outer pouch. If a requisition is contaminated by blood, the testing technologist must discard it.
5. Clean any spills immediately with a 6% sodium hypochlorite (Javex) solution.
6. Transport the specimen to the laboratory with any special treatment, like a hot water bath or ice, or store it safely. Do not leave the specimen where someone unscrupulous can tamper with it.

### Errors that would invalidate the test results:

- Misidentifying the patient
- Fasting status unchecked
- Ignoring legal requirement to identify yourself and explain the procedure to the patient
- Choosing the wrong equipment or anticoagulant
- Not allowing disinfectant to dry before collection
- Bevel down cannula insertion
- Collecting with an IV still running
- Excessive use of a tourniquet, and not removing it as the last tube fills
- Wrong order of draw
- Not milking the tubes enough to mix the blood properly with anticoagulant, or shaking them so hard that the cells hemolyze
- Hemolysis from rough handling (pulling hard on the syringe plunger or pushing the blood too quickly through a tiny needle into the receiving tube)
- Mislabeling tubes/Ignoring infection control protocol
- Omitting information from the requisition (tests, date, time, initials, comments)
- Delaying transport to the lab
- Storing specimens incorrectly
- Transporting specimens without the required additives, hot water, or ice

### **Places to avoid for collection**

You must not aggravate the patient's condition or slow down the healing process through blood collection. Avoid: Areas with circulation problems (varicose veins, cyanosis); blanched (frost nip) or darkened (frost bite) cold areas; blisters; broken skin (lacerations, abrasions, puncture wounds); bruises (hematomas, contusions); drawing so much blood as to cause anemia; edema; hardened skin (scleroderma, calluses, muscle spasms); infected

areas (hot, red, swollen, oozing pus); mastectomy side; paralyzed limbs, if possible; rashes, moles and discolorations; skin pricking immunosuppressed or leukemia patients – choose a venipuncture, with povidone swab and sterile gauze, instead; surgical sites (external pins, skin grafts, sutures); thinned skin (“steroid skin”); toes and fingers on infants – use the heel.

## **Hematology specimens**

Most rejected hematology specimens are clotted. Pop the stoppers on EDTA (lavender) or citrated (light blue) tubes and use wooden heparinized sticks to examine the rims for clotting. Clots indicate the tube was not mixed by inverting it end-to-end for 8—10 times immediately after collection. Clots may clog the testing machinery, and will cause inaccurate platelet results. The hematology specimen’s label must be well-attached, in indelible ink so that it cannot be erased, and legibly printed with the patient’s full name, identification number, collection date and time, doctor’s name, and tests requested. Make smears within 2 hours. Adult EDTA tubes must be Coulter tested within 6 hours of collection, and pediatric tubes (Microtainers) must be tested within 4 hours. Keep them both at room temperature. Check the blood/anticoagulant ratio is within 10% of the correct volume. Overfilled tubes (from pouring instead of venipuncture) clot. Underfilled tubes have crenellated red cells, low Hct and RBC, and don’t stain properly.

## **Storing hematology blood collection tubes**

Keep EDTA (lavender stoppered) and citrated tubes (light blue) at room temperature (39°F—77°F) before use. If the room is too hot, the vacuum may decrease, and the blood draw will be insufficient. Do not chill tubes before the collection unless the test specifically asks for this. If the tube is chilled after collection, then it must be tested before 24 hours elapse. Be aware that results may not be accurate because of clotting or hemolysis. EDTA chelates calcium ions to stop blood from clotting. Chelation may cause IgG autoantibodies to bind together, and the blood cell membranes to change. Platelets will form clots. Platelet satellitism may give a false increase in an automated cell count. If there is a problem with the patient’s platelets in an EDTA tube, order the CBC repeated on a blue citrated tube, which will not have the platelet reaction.

## **Correct order of draw**

The order of draw for venipunctures and for capillary punctures and the best locations for venipunctures and lancet punctures:

- *The correct order of draw for venous blood collection is:* Blood culture bottles; light blue sodium citrate tubes; serum tubes (plain or SST); green heparinized tubes; lavender EDTA tubes; and grey fluoride tubes. This draw will reduce the chance of contaminating and mixing anticoagulants. Venipunctures are best in the antecubital fossa, from the median vein or cephalic vein. If the antecubital is not suitable, try the dorsum of the wrist or the back of the hand.

- *The correct order of draw for capillary puncture is:* EDTA first; other colors next, and serum tubes last. The draw is different because capillary blood is more likely to clot or hemolyze during collection. Newborn capillary puncture is done with a short point lancet on the lateral or medial plantar heel surface to avoid nicking the bone and causing osteomyelitis. Older infants can have the plantar surface of their big toes pricked with a short point lancet. Children and adults have the distal phalanx palmar surface pricked with a long point lancet.

## **Urine samples**

The types of urine samples used by labs are: 24-hour; catheter; clean catch; fasting; first morning; midstream; postprandial; random; and timed. Labels and requisitions are legal documents. They should be in indelible ink, so that they cannot be changed, and will not blur if the ink comes in contact with urine. They must have the patient's name, date, time, physician's name, type of test requested, and any relevant medical history (e.g., diabetic, menstruating, porphyria). Random drug tests require *chain-of-custody* precautions to ensure specimen integrity. The specimen collection may be *witnessed* to prevent tampering, switching samples, or poor collection technique that could cause a false result. The porter must sign to guarantee *safe transport* to the lab. The lab must *time stamp* the requisition at Receiving. *Safe storage* and *careful analysis* guarantees are required in case the result is contested and it needs to be re-tested later.

### Random urine sample

Random urine is used at any time of day for a routine check-up, in any clean container that is free of contaminants. Random urine does not require a sterile container. It does not require a lid if it will be tested immediately, only if it will be transported or stored. The doctor may ask the patient to use a fine mesh strainer over the mouth of the container to isolate stones less than 5 mm in diameter that pass through the urinary tract unaided. Infant random urine collectors are small plastic bags that are attached to the baby's groin with a gentle adhesive; pour the bag contents into any clean, lidded container. Collecting and testing hands should be clean and free of contaminants that could affect the results. Store in a refrigerator between 2—8°C up to 72 hours. Note that the sample degrades if stored at or above 4°C and after 2 hours. The Chemistry Department tests random urines. Routine check-up tests include: Blood, protein, glucose, ketones, and pH.

## **Bone marrow**

A bone marrow biopsy should be drawn from the patient's hip in the iliac crest because it is easy to access and usually the safest. The hip bone is close to the skin's surface. No large blood vessels or nerves are nearby the collection point, so bleeding and pain are minimized. Battlement scan pattern produces a more standardized review of the slide than the wedge push technique because the cells are more evenly distributed across the slide in the battlement technique. In the wedge push, leukocytes pool in different sections of the slide: On the side edges and in the feather, eosinophils, monocytes, and segmented

neutrophils tend to concentrate, while small lymphocytes tend to concentrate in the center of the film.

## Media types

Some common types of media and the types of pathogen each is used to culture:

- *BACTEC* for mycobacteria
- *BCYEa* buffered charcoal yeast extract agar for Legionella
- *Bile-esculin* agar for Enterococcus and fecal strep
- *Bio-Bag* blood agar for gonorrhea, meningitis, haemophilus, and strep in CO<sub>2</sub>
- *Brilliant Green* agar for salmonella, except typhoid and paratyphoid
- *CDC* agar for anaerobes
- *JEMBEC* agar plate with CO<sub>2</sub> tablet for gonorrhea
- *Kligler Iron Agar (KIA)* for enterics
- *Lowenstein-Jensen (LJ)* egg media for tuberculosis
- *M* broth for Salmonella
- *MacConkey* agar (MAC) for gram-negative bacilli
- *Modified Wadowsky-Yee (MWY)* media for Legionella
- *Semisolid* agar replaces the hanging-drop test for motility (salmonella)
- *Mueller-Hinton* agar for Neisseria and disk-agar diffusion for susceptibility
- *Septi-Chek* blood culture media for septicemia
- *Sheep blood* agar for fastidious, slow-growing anaerobes
- *SP-4 Diphasic* broth for mycoplasma culture
- *Tinsdale* media for *C. diphtheriae*

## Organizations and regulations

### DOT and IATA

The Federal Department of Transportation (DOT) regulates shipping diagnostic and biological specimens, infectious substances, and anything shipped on dry ice. Follow standards set by the International Air Transport Association (IATA), regardless of the distance you are shipping, or the transportation method (truck, rail, air, boat). Label your shipment with one of these risk groups:

- *Risk Group 1:* Unlikely to cause human disease to an individual or community, e.g., stool smear for occult blood mail-in card.
- *Risk Group 2:* Can cause disease in an individual, but probably not throughout a community. Prevention and treatment are readily available, e.g., stool for O&P, and genital swab for candida albicans.
- *Risk Group 3:* Can cause serious disease for an individual, but probably not a community. Prevention is less readily available or treatment is less effective, e.g., a brain specimen for Creutzfeldt-Jakob (mad cow) disease, or a tube of blood for HIV test.
- *Risk Group 4:* Can cause serious disease for both individuals and the community. Prevention and treatment are not available, e.g. tuberculosis, encephalitis.

## Biologicals

A biological is a vaccine, diagnostic reagent, or derivative of a living organism intended to help prevent, diagnose, or treat a disease. If you are shipping vaccines, reagents, or research materials, you need to classify them as A, B, or C:

- *A*: Contains Risk Group 2 or 3, but probably not 4. Shipment almost certainly contains pathogens. Requires a first or second opinion (confirmatory diagnosis). A diphtheria swab fits this class.
- *B*: Small chance of harboring Risk Group 2 or 3. Specimen almost certainly does not contain pathogens. Routine screening or first diagnosis. A routine Pap smear fit this class.
- *C*: Shipment does not contain any known pathogens. Killed vaccines fit this class.

You need the IATA Blue Pages in order to fill out the shipping manifest with the IATA shipping name, class, subsidiary risk and UN codes. You must have a Class 6.2 DOT sticker, and if the biological is preserved with dry ice, a Class 9 DOT sticker.

## IATA Packing Instruction 650

Pack no more than one liter of blood product per shipment. Place the blood product in a glass, metal, or plastic watertight primary receptacle with a screw-on, snap-on, or push-on lid. Tape over it. Individually wrap each primary receptacle with absorbent cushioning material (cellulose, cotton balls, paper towels, or super-absorbent packet). Place the primary receptacle in the secondary receptacle, made of at least one inch thick Styrofoam, or plastic. The manufacturer must certify the secondary container as leakproof and siftproof. Both primary and secondary receptacles must withstand 95 kPa pressure and temperatures ranging from -40°F to +130°F. Place a list of contents between the secondary and outer packages. Use a strong outer package of corrugated cardboard, metal, wood, or plastic. Do not overload it. It must be able to survive a four foot drop. Mark "EXEMPT HUMAN SPECIMEN", "BIOLOGICAL SUBSTANCE CATEGORY B" and the phone number of the responsible person at least 6 mm high on the outside. The UN shipping code 3373 must appear inside a diamond shape.

## Epidemics, pandemics, and nosocomial cases

Epidemics, pandemics, and nosocomial cases, and how to ship specimens safely to a reference laboratory to avoid the spread of disease:

- *Epidemic*: Sudden disease outbreak in a limited community, with more victims at the same time than normally associated with that disease.
- *Pandemic*: An epidemic that becomes worldwide, e.g., Hong Kong Flu.
- *Nosocomial infection*: Secondary infection developed at least 48 hours after hospital admission, or within 30 days after discharge, e.g., bacterial pneumonia.
- You are responsible for the specimen shipment until the reference lab takes possession of it, and will incur fines for improper shipping. Use the IATA Blue Pages

to complete the form with: Authorization; contents; Class 6.2 DOT sticker; if shipped on dry ice, a Class 9 DOT sticker and ice weight; IATA shipping name, class, subsidiary risk and UN codes; most responsible person's name; most responsible consignee's name; packer's name; shipper's name, and declaration if it is a restrictive specimen; quantity and type of packaging, packing instructions, place and time of packing; street address, city, and zip code of origin and destination; 24-hour emergency landline telephone number attended by a responsible person who can contact an expert immediately.

## **Chain of custody**

Chain of custody is the legal procedure to ensure integrity of a specimen. A special form is used to document this workflow:

- Initial specimen collection, which may require a witness to prevent tampering, switching samples, or poor collection technique that could cause a false result
- Safe transport to the lab
- Receipt by the lab (time stamp)
- Safe storage of the specimen (especially if the result is contested and it needs to be re-tested later)
- Careful analysis of the specimen

Chain-of-custody is crucial for pre-employment drug testing and forensics. For example: *A train engineer is subjected to a witnessed urine collection to ensure he/she is drug-free as a condition of employment.* Poor collection technique would be if the COLT does not ask if poppy seeds were eaten recently before the test, resulting in a false positive result for heroin. Another example would be when a driver suspected of driving under the influence (DUI) has a high blood alcohol level drawn for a court case, but her friend substitutes a negative sample.

## Systeme Internationale d'unités

On May 20, 1875, the U.S.A. signed the Treaty of the Meter at the International Bureau of Weights and Measures in Paris, France. American scientists joined colleagues in 48 countries by measuring in Systeme Internationale (SI) units, for the sake of clarity and consistency: Ampere (electrical current); Candela (light); Kelvin (temperature); Kilograms (mass); Meters (distance); Mole (substance); Seconds (time).

The U.S.A. and the United Kingdom are the only two countries that still allow two sets of measurements for lab tests, but they are phasing out conventional units in favor of SI. The U.S. National Institute of Standards and Technology (NIST), encourages labs to convert to SI. Here are some normal values converted from conventional units to SI units:

Normal Values		
Test	Conventional units	SI units
Hemoglobin	12 to 16 g/dL	7.4 to 9.9 mmol/L
Albumin	3.2 to 4.5 g/dL	35 to 55 g/L
Amylase	56 to 190 IU/L	111 to 296 U/L
Bilirubin	0.1 to 0.3 mg/dL	1.7 to 5.1 $\mu$ mol/L

### Differences in reference values

*Normal reference values* are usually within 2 standard deviations of the mean for the normal patient population. A particular laboratory's reported normal values may differ from others' because:

- Norm was based on a fasting sample, rather than a random sample
- Patient's posture was different (supine or standing)
- Tourniquet was used or omitted
- Averaged results for their patient population were skewed by age, race, drugs, exercise, collection time, etc.
- Normal value was reported for a nutritional ideal few can meet in deprived areas
- Equipment was different
- Test method was different
- Collection was different
- Storage was different
- SI units were used instead of conventional U.S. measurements

*Margin of error* is the statistical chance (confidence level) that some tests will be abnormal because of strict reporting limits. For example, if a lab test is accurate up to the 95<sup>th</sup> percentile, then five out of every 100 patients tested will have abnormal results, even though those five patients are actually healthy.

# Chemistry and Urinalysis Tests

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## Chemistry

### False readings and results

#### Pseudohyperkalemia, hyperammonemia, and falsely decreased bilirubin

Pseudohyperkalemia is a false reading of increased serum potassium. It can be caused by poor venipuncture technique or transfusing hemolyzed blood. Hyperammonemia is elevated ammonia level in the blood, affecting pH. Tightening the tourniquet too much during venipuncture, storing the tube too long before testing, or hemolyzing the sample cause the ammonia level to be artificially high. The false result may give the impression the patient has a serious digestive disorder. Mark on the requisition anything else that would elevate the test: Smoking, not fasting for at least 8 hours before collection, or heavy exercise. The CLT can cause an artificially lowered bilirubin count by leaving a specimen tube exposed to light at room temperature for one hour; shaking the specimen or allowing air bubbles in it; not warning the patient to avoid antibiotics, antimalarials, barbiturates, diuretics, fasting more than 8 hours, fatty meals, orange vegetables (carrots and yams), salicylates, and steroids before the test.

#### Instances which cause a false result:

- *Drawing blood from an arm with an IV:* Dextrose IV can lower the serum amylase level.
- *Allowing bubbles in a specimen tube:* Bubbles in an arterial blood gas syringe can increase the gas levels; bubbles in a venous bilirubin sample can decrease the bili level.
- *Not icing a sample immediately:* Blood gases are untestable if they are kept at room temperature, even for a few minutes, because ice slows down cellular respiration.
- *Using the wrong anticoagulant:* Using any anticoagulant other than oxalate in a grey stoppered tube causes an artificially low blood sugar, because the cells continue to eat glucose in the tube.
- *Scheduling an examination before a blood test:* Scheduling a prostate exam or TURP procedure less than one week before a PSA test can artificially increase the PSA to indicate prostate cancer.
- *Failing to ask a patient if he/she has pre-existing conditions before a test:* Failing to ask a patient if she has had an amniocentesis, a history of cirrhosis, or hepatitis could result in misdiagnosis from an artificially high AFP level. Failing to ask a patient if she is pregnant could invalidate a Beta-HCG level.
- *Mislabeling a sample:* Mislabeling a semen sample could lead to an incorrect diagnosis of infertility.



## Cardiac enzyme studies

The cardiac enzyme studies, their acronyms, normal values, and changes over time after a heart attack (MI):

<b>High cardiac enzymes indicate heart muscle damage</b>			
Chemical Name	Acronym	Normal	Significance
Creatine phosphokinase and Creatine kinase	CPK and CK fractionation	5 to 75 mU/mL	Rises 6 hours after heart attack, peaks in 18 hours, and returns to baseline in 3 days. Rise of CK-MB means severe damage.
Serum aspartate aminotransferase	AST	12 to 36 U/mL	Rises 6 to 10 hours after heart attack, peaks at 12 to 48 hours, and returns to baseline in 4 days.
Lactic dehydrogenase:	LDH	90 to 200 ImU/mL	Rises 24 to 72 hours after heart attack, peaks in 4 days, and returns to baseline in 14 days.

## Myocardial infarction misdiagnosis

A rise in cardiac enzymes does not always indicate heart muscle damage. There are other conditions that could be misdiagnosed as a myocardial infarction (MI) because of an enzyme rise. High cardiac enzymes do not necessarily mean a heart attack. CPK can be elevated if there is alcoholism that is so chronic there is muscle damage (myopathy); cardiac catheterization to diagnose vessel disease; cerebrovascular accident (CVA); Clofibrate use; electric shock applied to restart the heart (cardioversion); hypothyroidism (high TSH, low T<sub>3</sub>, T<sub>4</sub>); surgery. AST can rise because of anticoagulants (Coumadin); anti-inflammatories (Aspirin); burns; Central Nervous System damage; crushing injuries; hemolytic crisis (Sickle cell anemia); infarction of intestines, kidneys, or spleen; liver disease; myopathy; opiates (heroin, OxyContin); pancreatitis; toxemia of pregnancy. LDH can rise because of congestive heart failure and lung disease.

## Hemoglobin, bilirubin, and iron

When the liver destroys old blood cells at the end of their 120 lifespan, it uses the freed-up hemoglobin to make bile, which it stores in the gall bladder, and the small intestine uses to break up fats. Normal adult total bilirubin is 0.3 to 1.0 mg/dL. If RBC's are destroyed too quickly, or if the liver cannot excrete excess bilirubin, there will be a rise above 1.0 mg/dL and the patient may look jaundiced (yellow eyes and skin, brownish urine and pale feces). The total bilirubin can be divided into direct or conjugated, rises in which indicate gall bladder stones, Dubin-Johnson syndrome, or cancer, and indirect or unconjugated, which rise in hematomas, infarcted lungs, or Gilbert's disease.

RBC manufacture requires iron, protein, B<sub>12</sub> and folate. The liver stores iron as ferritin protein or hemosiderin when it breaks down the old RBC's for reuse later. Hemochromatosis and beta thalassemia (Cooley's anemia) patients have an inherited problem of storing too much iron, which destroys their internal organs and makes them jaundiced.

## Liver function enzymes

The three main liver function enzymes:

- *Alkaline Phosphatase (ALP) Normal Range:* 30 to 85 IU/mL Adult and up to 300 in children with growing bones. *Significance:* Used to differentiate between bone and liver diseases. Increases in diseases of the bile duct, bone fractures and Paget's disease, liver cancer, hyperparathyroidism, hyperphosphatasia, infarcted bowel, and rheumatoid arthritis. Decreases in pernicious anemia, celiac disease, hypothyroidism, low serum phosphorous (hypophosphatemia), malnutrition, especially scurvy, and high levels of B vitamins.
- *Serum Alanine Aminotransferase (ALT) Normal Range:* 5 to 35 IU/L. *Significance:* Increases in liver disease. AST: ALT ratio should be 1:1. If it is greater than 1:1, it can mean cirrhosis, congestion, or tumors of the liver. If the ratio is less than 1:1, suspect hepatitis or mononucleosis.
- *Serum Aspartate Aminotransferase (AST) Normal Range:* 5 to 40 IU/L. *Significance:* Increases in beriberi, burns, diabetic ketoacidosis, heart damage (MI), hemolytic anemia, kidney disease, liver damage, myopathy, pancreatitis, pregnancy, skeletal disease, and traumatic injury.

## Newborn jaundice

Newborn babies have more RBC's than adults, many reticulocytes, and immature livers that are not yet efficient at breaking down bilirubin.

- *Erythroblastosis fetalis:* Babies whose Rhesus factor is incompatible with their mothers' may develop hemolytic disease of the newborn, and are jaundiced within two days after birth because their RBC's are being destroyed. They require blood transfusions.
- *Kernicterus:* Hyperbilirubinemia over 5.0 mg/dL can mean the baby has congenital icterus (weak RBC walls) that leads to hemolytic anemia. The baby may be limp (hypotonia) and tired, then have seizures, and may become deaf, mentally retarded or develop cerebral palsy if left untreated. Phototherapy turns bilirubin in the skin that causes jaundice into lumirubin that the baby can excrete in urine. Extreme cases require blood transfusions.
- *Physiologic Jaundice:* Breastfed babies released from the hospital early, especially if they do not receive a Vitamin K injection, tend to become jaundiced, but this is normal and resolves in approximately one week by itself, as long as fluid intake is adequate.

## Liver and kidney relationship

There is a relationship of the liver and kidney as they regulate wastes from protein metabolism. Ammonia, BUN, creatinine, and uric acid are waste products of protein metabolism. The liver cleans ammonia from protein catabolism by changing it into urea to maintain the pH balance of the body, and the kidneys excrete urea in urine to protect the brain. Normal adults have 15 to 110 mcg/d of ammonia; children have higher values. Hyperammonemia can mean the patient has cirrhosis, liver coma, gastrointestinal bleeding or stasis, kidney disease, or Reye's syndrome. The patient may also be taking TPN (Total Parenteral Nutrition) because of a digestive problem. Decreased ammonia may be from kidney failure, malignant hypertension, and using tetracycline or neomycin. Creatinine and BUN are renal function studies. High Creatinine (over 1.5 mg/dL) and Blood Urea Nitrogen (over 20 mg/dL) means the patient has kidney diseases like glomerulonephritis, pyelonephritis, stones, tubular necrosis, or tumors. BUN drops when using Tobramycin or Gentamycin, in congestive heart failure, dehydration, liver failure, pregnancy, and shock.

## Pancreatic enzymes and pancreatitis

The main enzymes the pancreas secretes are: The proteases chymotrypsin and trypsin that digest proteins into peptides; carboxypeptidase that breaks peptides into amino acids; lipase that breaks down triglycerides; amylase that breaks starch to maltose. Minor enzymes the pancreas secretes are elastase, deoxyribonuclease, gelatinase, and ribonuclease. Pancreatitis is inflammation of the pancreas where the enzymes are trapped and digest the pancreas. Acute pancreatitis is usually from cholelithiasis or alcoholism, and can be cured with total parenteral nutrition (TPN) for about a month and abstaining from alcohol. Chronic pancreatitis can also be from alcoholism, but is more likely inherited and starts in childhood from cystic fibrosis, pancreas divisum, hypercalcemia, hyperlipidemia, or autoimmune diseases. Chemistry blood tests for pancreatitis and their normal values are amylase (50—190 IU/L), lipase (0—100 u/L), and trypsinogen (<140 mcg/L). The stool tests for pancreatitis are fecal fat and trypsin. Sweat is collected on filter paper and analyzed for sodium and chloride to rule out cystic fibrosis.

## Serum tests

The following serum tests and their normal values:

- *Amylase* helps to diagnose pancreatitis, alcoholism, ectopic pregnancy, hepatitis, and diseases of the intestine, kidneys, and salivary glands. Amylase is normally 56 to 190 IU/L.
- *GGT* is mainly used to diagnose alcoholism (75% of alcoholics have high levels), but it can also show an increase 4 to 10 days after a heart attack, or if the patient is taking phenobarb and Dilantin. When the patient has a high ALP, the gamma-glutamyl transpeptidase can help differentiate bone from liver disease, especially in children and pregnant women. Male GGT should range from 8 to 38 U/L and women under 45 should range from 5 to 27 U/L.

- *LAP* is mainly used to diagnose cancer of the liver that has metastasized, and gall stones, but it can also help to pinpoint a diagnosis when ALP is high.
- *5'-NT* detects cancer that has metastasized when the patient is not jaundiced, and helps to differentiate bone from liver disease, and gall bladder disease in pregnant women. Normal value is 0 to 1.6 units.

## Diabetes tests

The common tests for diabetes mellitus:

- *C-peptide* tests for hypoglycemia, insulin production in the pancreas, insulinoma tumor, and unauthorized insulin injections.
- *Glucagon* encourages the liver to free up glucose to balance insulin in normal patients. Diabetics, or patients with high catecholamines, have high glucagon levels. Low glucagon levels could mean part of the pancreas is destroyed.
- *Glucose* is collected fasting (FBS), random, 2 hours p.c. (postprandial), one-hour pregnancy challenge (GDM), or three or five hour tolerance (GTT). Normal fasting is less than 110 mg/dL, peaks two hours after eating, and then drops precipitously. It needs a grey stoppered tube because the oxalate stops the blood cells from eating sugar.
- *Hemoglobin A<sub>1C</sub>* tests for glucose bound to hemoglobin in red blood cells over their lifespan. It is a snapshot of how the patient's diabetic medication has controlled his/her hyperglycemia for the past three months.
- *Insulin* is manufactured by the Islets of Langerhans in the pancreas and it regulates blood sugar. It is measured to mark insulinoma tumors and hypoglycemia. Insulin panic value is more than 35  $\mu$ IU/mL.

## Cortisol levels

Cortisol is a stress hormone produced by the adrenal glands. It is drawn in the morning and afternoon to see if it follows a normal daily rhythm (circadian). Cortisol is normally higher around 8:00 in the morning (6 to 28 mcg/dL) and lower at 4:00 in the afternoon (2 to 23 mcg/dl), called diurnal variation. In-patients should have very low nocturnal cortisol around midnight because they are resting and not under stress. Patients with Cushing's syndrome do not have a normal circadian rhythm for their cortisol – it starts off high in the morning, and stays high in the afternoon. Patients with Addison's disease have low cortisol levels and need to also have a 24-hour urine test for 17-Hydroxycorticosteroids. Abnormal cortisol levels can also occur in patients whose thyroid and pituitary glands are malfunctioning, who are obese, are taking steroids, diuretics, and birth control pills, or who have cancer.

## Aldosterone and androstenedione

Patients who urinate frequently, have high blood pressure, muscle cramps and weakness have morning and evening venipunctures and 24-hour urine collections for *aldosterone*, an

adrenal hormone that works with renin and should measure between 1 and 21 ng/dl. They could have Bartter's, Conn's, or Cushing's syndromes, or toxemia.

Women with male pattern hair growth (hirsutism) may have too much *androstenedione* produced by their ovaries, ovarian cysts, virilizing tumors, or adrenal problems like Cushing's syndrome. If more than 1,000 mg/dl of androstenedione, suspect a tumor.

### **Atrial natriuretic factor tests**

The heart's atria produce ANF when they are overloaded with blood volume and high blood pressure. Patients may be short of breath, cough, be lethargic, and have edematous legs. They could have paroxysmal atrial tachycardia. ANF blocks ADH and aldosterone to encourage loss of water and salt that relieves the pressure. Too much sustained pressure can eventually lead to congestive heart failure. ANF should be 20 to 77 ng/L.

### **Electrolytes**

Electrolytes are electrically charged salts that make your muscles and nerves work, especially your heart. They are referred to as "lytes", and it is common to write them in the chart by their chemical notation, shown in brackets. An electrolyte panel includes: Bicarbonate ( $\text{HCO}_3$ ) that is usually tested as carbon dioxide ( $\text{CO}_2$ ), Chloride ( $\text{Cl}^-$ ), potassium ( $\text{K}^+$ ), and sodium ( $\text{Na}^+$ ). The other electrolytes, usually classified as part of a mineral panel, are: Calcium ( $\text{Ca}^{2+}$ ), Magnesium ( $\text{Mg}^{2+}$ ), and Sulfate ( $\text{SO}_4^{2+}$ ). Electrolyte levels are controlled by your kidneys, perspiration, and dietary intake, so they will be lost by the patient with vomiting and diarrhea or bulimia. According to the Center for Disease Control, about half a million children worldwide die each year from severe diarrhea and vomiting caused by rotavirus. Excessive loss of electrolytes damages the heart and kidneys. Normal serum concentration ranges are: Carbon Dioxide: 23 to 30 mEq/L; Chloride: 90 to 110 mEq/L; Potassium: 3.5 to 5.0 mEq/L; Sodium: 126 to 145 mEq/L.

#### These terms associated with the electrolytes:

- *Hypernatremia*: Increased sodium concentration in the blood serum. It can be from: Burns; Cushing's syndrome; dehydration; diabetes insipidus; glucosuria (brittle diabetics lose a lot of urine); hyperaldosteronism; IV fluid that is too concentrated; salty diet.
- *Hyponatremia*: Decreased sodium concentration in the blood serum. It can be from: Addison's disease; ascites; congestive heart failure; diarrhea and vomiting; diuretics like Lasix; edema; ileus; kidney insufficiency where the tubules do not reabsorb correctly; pleural effusion; sodium-restricted diet; water retention from too much antidiuretic hormone (ADH); water toxicity; IV fluid that is too diluted.
- *Hyperkalemia*: Increased potassium concentration in the blood serum. It can be from: Acidosis; Addison's disease; crushing injuries; diuretics like spironolactone; hypoaldosteronism; infection; kidney failure; IV fluid that is too concentrated.
- *Hypokalemia*: Decreased potassium concentration in the blood serum. It can be from: Alkalosis; stomach cancer; Cushing's syndrome; diarrhea and vomiting;

diuretics; eating too much licorice; hyperaldosteronism; calcium, insulin or glucose injections; renal tubular acidosis.

### Arterial blood gases

Out-patients are rarely checked for arterial blood gases. Arterial blood gases check an in-patient for: Acidosis (the patient has respiratory depression because of drugs or CNS trauma, or has metabolic problems because of diabetes, kidney failure, fistula of the intestine, or shock); alkalosis (the patient is hyperventilating because of severe pain and emotional upset, or has metabolic problems because of prolonged vomiting, or a nasogastric feeding tube that is draining incorrectly); congenital heart disease; cor pulmonale (right heart failure); pneumonia; pulmonary embolism. Oxygen saturation should always be 95 to 100% in normal patients. In a sleep lab, arterial blood gases may be drawn to diagnose obese patients with Pickwickian syndrome, where they breathe very shallowly and have sleep apnea, so that their carbon dioxide is always high, and their oxygen saturation is low.

Test	Normal Values	Panic Values
pH	7.35 to 7.45	Less than 7.20
PO <sub>2</sub>	80 to 100 torr	Less than 50 torr
PCO <sub>2</sub>	35 to 45 torr	More than 60 torr

### Drug level panic values

The panic values for these drug levels:

Drug	Use	Panic Value
Digoxin	Slows atrial fibrillation and flutter.	More than 4.0 ng/mL
Dilantin or Phenytoin	Controls epileptic seizures.	More than 2.5 mcg/mL
Digitoxin	Used like digoxin for patients with poor kidney function, because it is excreted by the liver.	More than 40 ng/mL
Disopyramide or Norpace	Slows SA node and controls ventricular arrhythmias.	More than 7.0 mcg/mL
Lidocaine	Anesthetic and controls ventricular arrhythmia and tachycardia.	More than 6.0 mcg/mL
Procainamide	Treats premature ventricular contractions, supraventricular arrhythmias, and tachycardia.	More than 10 mcg/mL
Quinidine	Treats malarial fever and reduces and prevents arrhythmias.	More than 6 mcg/mL
Theophylline	Treats asthma, bronchitis, and emphysema.	More than 20 mcg/mL

## Cholesterol and triglyceride

Cholesterol is lipids from animal sources that climb after a fatty meal. To obtain a fair reading, total cholesterol should be tested after a 12—14 hour fast, when it should read 150 to 250 mg/dl. While a high total cholesterol level suggests heart disease and atherosclerosis jaundice from gall bladder obstruction, and brittle diabetes, it can be very misleading without a fractionation into High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL). A low total cholesterol level may look reassuring, but can mask diseases like celiac disease with fat malabsorption, goiter, and liver disease. If the proportion of HDL (at least 55 mg/dl) is higher than LDL, it significantly decreases the patient's chances of a heart attack. If the LDL (more than 180 mg/dl) is of higher proportion than HDL, it means the patient has an increased chance of coronary artery disease requiring a bypass. Triglycerides are lipids from carbohydrate and alcohol sources. They should be no higher than 150 mg/dl. Hi "trig" is also a heart risk.

### Drugs affecting the following:

- *Amylase* can be increased by birth control pills, codeine, cholinergics (Tensilon, Humorsol), ethyl alcohol, methacholine for asthma tests, and pain killers like morphine and meperidine.
- *Bilirubin* can be increased by birth control pills, antimalarials, Aspirin, cholinergics, coumarin, morphine, penicillin, Quinidine, Rifampin to treat tuberculosis, and antibiotics like streptomycin, tetracycline, and sulfas. Bili decreases with barbiturates, corticosteroids, phenobarb, and the anti-psychotic, thioridazine.
- *Calcium* can be increased by antacids (Tums), estrogen, birth control pills, secretin, anabolic steroids, thiazide diuretics, and Hydralazine (antihypertensive). Calcium decreases with corticosteroids (Prednisone), mercurial diuretics, insulin, Phenytoin, sulfates, and gastrin.
- *Chloride* can be increased by marijuana, corticosteroids, phenylbutazone, and guanethidine antihypertensive. Chloride decreases with aldosterone, baking soda, cortisone, prednisolone eye drops, diuretics and laxatives.
- *Cholesterol* can be increased by anabolic steroids, birth control pills, cinchophen, cortisone, epinephrine, Phenytoin, pregnancy, sulfa drugs, and thiazide diuretics. Cholesterol decreases with the fertility drug chlomiphene, the lipid-lowering drug Clofibrate, antibiotics like erythromycin, neomycin, and tetracycline, garlic, and MAO antidepressants.
- *CO<sub>2</sub>* can be increased by aldosterone, baking soda (sodium bicarbonate), hydrocortisone, laxatives, diuretics, Prednisone, and Viomycin. CO<sub>2</sub> decreases with antibiotics like methicillin, nitrofurantoin, and tetracycline, the glaucoma medicine acetazolamide, the anticonvulsant dimethadione, the antiglycemic phenformin, and the diuretic triamterene.
- *Glucose* can be increased by Aspirin, caffeine, cortisone, dopamine, ephedrine, epinephrine, estrogen, furosemide, Levodopa, phenylbutazone, and Phenytoin. Glucose decreases with insulin, erythromycin, diuretics, and sulfas.
- *Potassium* can be increased by epinephrine, heparin, histamine, marijuana, the diuretic spironolactone, and the antibiotics Amphotericin B, methicillin, and

tetracycline. Potassium decreases with aldosterone, Aspirin, sodium bicarbonate, cortisone, most diuretics, insulin, licorice, and the antibiotics furosemide and Gentamycin.

- *Protein* can be increased by anabolic steroids, androgens, corticosteroids, digitalis, epinephrine, insulin, and thyroid medications. Protein is decreased by estrogen and birth control pills.

## Tumor markers

Four common tumor markers:

- *Alpha-fetoprotein*: AFP detects liver and testicular cancers. It can be increased by amniocentesis, birth defects, cirrhosis, and hepatitis. Any level over 11 mcg/L needs investigation.
- *Carcinogenic Embryonic Antigen*: CEA appears in all cancers, so it is a generalized test for monitoring the progress of any cancer, but it cannot differentiate which organ is affected. Any level over 4.0 mcg/L should be investigated further.
- *Human Chorionic Gonadotropin Beta subunit*: Beta HCG detects choriocarcinoma, hydatiform moles, and germ cell tumors of the ovaries and testes. It is invalid during pregnancy and is increased in many other cancers. Normal values are less than 5 IU/L.
- *Prostate-specific Antigen*: PSA detects recurrent cancer of the prostate. It increases in 80% of men with prostatic cancer, but may also increase if there is benign prostatic hypertrophy that just requires a TURP to “shell out” the overgrown prostate. PSA rises in men as they age. Any PSA result from 4.0 to 8.0 mcg/L should be investigated further with a rectal examination to palpate the prostate.

## Serum protein tests and protein electrophoresis

A serum protein test includes Total Protein, Albumin, and Globulin. Normal values are: Total Protein 6—8 g/dl, albumin 3.2— 4.5 g/dl, globulin 2.3— 3.4 g/dl. Low albumin can mean kidney disease (nephrotic syndrome if more than 3.5 grams are shed in the urine daily), liver disease (like cirrhosis or hepatitis) and ascites (extra fluid in the peritoneum from end-stage diseases of the heart, kidney, liver, ovary, and pancreas). Low globulin can indicate the patient has severe burns, malnutrition, kidney or liver disease. When serum protein indicates disease, the doctor usually follows up with protein electrophoresis, which adds four globulin fractionations to the Total protein and Albumin: Alpha<sub>1</sub>-globulin, Alpha<sub>2</sub>-globulin, Beta-globulin, and Gamma globulin. Electrophoresis patterns and history of drug use can help pinpoint the diagnosis, which may also extend to rheumatoid arthritis, muscle tumors, and immune deficiencies. Normal values for the globulins are: Alpha<sub>1</sub>-globulin 0.1—0.4 g/dl, Alpha<sub>2</sub>-globulin 0.5 —1.0 g/dl, Beta-globulin 0.7—1.2 g/dl, and Gamma globulin 0.5—1.6 g/dl.



## Vitamins

The fat-soluble and water soluble vitamins:

Vitamin	Soluble	Disease	Food Source	RDA
A	Fat	Night blindness	Carrots, liver, eggs	5,0000 IU
D	Fat	Rickets; osteomalacia	Fortified milk, liver, eggs	400 IU
E	Fat	Abnormal growth	Seeds, cereal oils, fish, liver, eggs	30 IU
K	Fat	Long clotting time	Green vegetables and gut bacteria	1 mg
C	Water	Scurvy	Citrus, tomatoes, strawberries	60 mg
B <sub>1</sub>	Water	Beriberi	Green vegetables	1.5 mg
B <sub>2</sub>	Water	Depression	Green vegetables	1.7 mg
B <sub>3</sub>	Water	Pellagra	Beans, peanuts, cereal, fish, meat	20 mg
B <sub>5</sub>	Water	Tingling hands; burning feet; grey hair	Mushrooms, sunflower seeds, yogurt, liver	10 mg
B <sub>6</sub>	Water	Convulsions	Beans, cereal, meat	2 mg
B <sub>12</sub>	Water	Pernicious anemia	Meat, fish	6 mg
Biotin	Water	Depression	Chocolate, eggs	0.3 mg
Folic acid	Water	Anemia; spina bifida in babies	Green vegetables, cereal, liver	0.4 mg

# Urinalysis

## Urine samples

### Common dipstick tests for urinalysis

- *Blood (negative)*: Intact or hemolyzed red blood cells indicate bleeding due to infection, menstruation, paroxysmal hemoglobinuria, or trauma.
- *Glucose (negative)*: Uncontrolled diabetics and women with gestational diabetes spill sugar into their urine when their renal threshold is exceeded.
- *Ketones (negative)*: Uncontrolled diabetics, extreme dieters, and starving people produce ketones in urine when their bodies burn fat instead of sugar.
- *Leukocytes (negative)*: White blood cells indicate bleeding due to infection.
- *pH (5 to 9)*: Acidic urine helps the bladder resist infection. Alkaline urine encourages bacterial growth.
- *Protein (up to 8 mg/dl)*: Albumin is shed from the kidneys if the nephrons are damaged. Trace protein can be from genitals or feces.
- *Nitrites (negative)*: Some bacteria, like E-coli, produce nitrites after eating nitrates, so this is an indicator of infection.

### Correctly storing and using dipsticks

Test urine within one hour of collection, or refrigerate at 2°C—4°C. Warm urine to room temperature before testing. Test delays give false-negative results because bacteria eat glucose in urine. Hypochlorite or peroxide contamination produces false-positive results. Centrifuging urine before dipsticking or touching reagent pads can cause false results. Wash and glove your hands. Take one strip from the container. Cap it immediately. Dip the strip quickly and completely in urine. Blot on paper towel to avoid reagents running together. Reagent pads change color according to the amount of substances present in the urine, so color blind technicians should not dipstick urine. Compare the reagent pads at the exact time listed on the label with the color chart. Do not touch the strip to the label. Store strips between 15°C—30°C in a dark, dry place. Heat, light, or moisture exposure degrades the strips. Keep tightly capped in the original bottle with desiccant packet. Do not combine different lot numbers in the same bottle. Use before the expiry date.

### Collecting and storing midstream urine samples (MSU) and streaking a plate

Collect midstream urine (MSU) any time of day. Use a sterile, lidded container and a benzalkonium chloride wipe. (If your pathologist bans wipes, it is a clean catch.) Use midstreams for Bacteriology to diagnose bladder and kidney infections. The patient cleans the urinary opening (meatus), voids a little urine, collects the midstream of urine, and voids the remainder in the toilet. Touching the inside of the container contaminates the specimen and produces a false-positive; touch the outside of the MSU container only. Wash and glove. Open an agar plate. Dip a sterile swab or loop in urine. Press the swab or loop lightly across the agar's surface to plant the bacteria for culture. Close the plate tightly. Label it with the patient's name, date and time, physician's name, and test (Urine for C & S).

If you are sending the specimen to a reference lab, refrigerate it until pick-up. If you are testing it on-site, place it in an incubator for 24—48 hours.

Parasites that can be found in urine:

- *Filariasis* is from threadworms carried by mosquitoes. It causes cyluria (lymphatic fluid in the bladder) because the worms block lymph drainage. The penis and scrotum enlarge with pooled lymph. It is prevalent in tropical countries.
- *Plasmodium falciparum* malaria parasites are injected by infected mosquitoes. They destroy the kidneys' glomeruli and hemolyze red blood cells, causing kidney failure. Infection is known as blackwater fever because the victim's urine is black.
- *Schistosomiasis* (bilharzia) is caused by flukes (flatworms). It can destroy the kidneys and cause cancer of the bladder. It is prevalent in Africa, Asia, and South America in fresh water infested with snails.
- *Trichomoniasis* is caused by protozoa in the genitals that cause yellow-green, foul-smelling froth and severe itching. Sometimes they colonize the bladder, prostate, and urethra.

#### Macroscopic urine examination

If you are examining a 24-hour urine sample macroscopically, note the quantity first. Normal urinary output is 750—2000 mL daily. Three important characteristics of any type of urine sample are: Color, clarity, and aroma. Normal color is colorless to straw to amber, according to concentration. Normal urine is clear. Normal urine is aromatic from volatile acids.

Many drugs discolor urine. Abnormal colors are:

- Red, indicating fresh blood or blood from the lower urinary tract, and probably protein in the urine, or eating beets
- Brown, indicating old blood or blood from high in the urinary tract, or eating rhubarb
- Dark yellow, indicating bile from the liver
- Green, indicating pseudomonas

Abnormal clarity is cloudiness (turbidity), indicating infection or protein loss from the kidneys. Foul smelling urine indicates a urinary tract infection. Urine that smells like honey, rotten fruit, or nail polish remover indicates ketoacidosis of diabetes.

#### Microscopic urine examination

Centrifuge 10—15 mL of fresh urine in a V-bottomed, parafilm plastic tube for 5 minutes at 3,000 rpm. Pour off (decant) the supernatant. Leave 0.5 mL in the bottom of the tube. Resuspend the sediment by shaking gently. Pipette a drop onto a glass slide. Place a coverslip over it. Examine microscopically under low power first; bacteria and some cells may need high power.

#### Artifacts and rejecting samples

Artifacts are vaginal squamous cells, sperm, cotton clothing fibers, and baby powder starch granules. Reject MSU for C&S if unrefrigerated >1 hour, not in a sterile bottle, or a random

sample. Unrefrigerated urine has bacterial overgrowth, increased turbidity and nitrite, ammonia increase leading to higher pH, disintegration of casts and cells in alkaline urine, and decreased glucose from bacteria eating it as they multiply. Reject urine samples exposed to light >1 hour because bilirubin degrades if not kept in a dark bottle, urobilinogen oxidizes to urobilin, and color changes. Aminolevulinic acid (used to detect lead poisoning and porphyria), glucose and metabolites might be reduced. Ketones might decrease if it was hot enough for volatilization. Without acid wash and/or acid added to the bottle, these chemicals degrade: Aldosterone, 5-HIAA, 17-Hydroxycorticosteroids, 17-Ketogenic steroids, 17-Ketosteroid, citrate, cystine, electrolytes, estriol, estrogens, FSH, glucose, LH, metanephrine, phosphorous, porphobilinogens, porphyrins, pregnanediol, pregnanetriol, urea nitrogen, and VMA. If the collection bottle has metal in it, these tests might be incorrect: Arsenic; cadmium; chromium; lead; magnesium and mercury.

### 24-hour urine for creatinine clearance

The 24-hour Creatinine Clearance test determines if kidneys are damaged by measuring their output of creatinine against the blood level. If the kidneys do not filter properly, creatinine output in the urine decreases, and blood levels increase. Normal male: 95 to 104 mL/min. Normal female: 95 to 125 mL/min. BUN to Creatinine ratio between 15:1 and 20:1 is a normal renal function study.

- *Patient Instructions:* Two days before and during the test, do not eat more than 8 oz. of red meat, take Vitamin C, drink caffeine, or exercise strenuously. Discard your first morning urine in the toilet. Note the start time. Collect all urine in the jug up to and including the same time next morning. Keep the jug on a bucket of ice or in the refrigerator. Note the time of your last collection. Return to the lab next morning to have your blood creatinine level drawn for comparison.
- *Drugs that affect the test:* Diuretics, Vitamin C, L-DOPA, Tagamet, Mefoxin, Dilantin, Garamycin, quinine, Procainamide, Amphotericin B, and tetracycline.

### Specific gravity test on urine

The specific gravity test (SG) shows the concentration of solids in the urine. The instrument used for testing SG is the urinometer. Normal range is 1.015 to 1.025. The highest specific gravity is found in the first morning sample, when concentration is greatest. Specific gravity for water is 1.000, so if urine is diluted by drinking, or if the kidneys are not filtering adequately, the SG will be nearer to water.

*SG increases* in patients who have:

- Protein or glucose in the urine
- Narrowed renal arteries that reduce blood flow to the kidneys
- Pituitary tumors that release antidiuretic hormone (ADH)

*SG decreases* in patients who have:

- Diabetes insipidus
- Chronic kidney failure (CKF)
- Too much water and not enough salt (hyponatremia)

### 24-hour urine test for VMA

24-hour urine test for VMA and Catecholamines is done to diagnose hypertension (very high blood pressure) due to pheochromocytoma (tumor of the adrenal gland that produces too much adrenaline). Sometimes it detects neuroblastomas, ganglioneuromas, and ganglioblastomas. It is more sensitive than a blood test for catecholamines. The normal value for VMA is 1 to 9 mg/24 hours. The catecholamines are: Epinephrine, norepinephrine, metanephrine, and normetanephrine. Patient Instructions: For three days before the collection, abstain from coffee, tea, bananas, chocolate, licorice, citrus, vanilla, aspirin, blood pressure medication, heavy exercise, and strong emotion. Void in the toilet at 8:00 a.m. Collect urine up to and including 8:00 a.m. the next day in the jug. Transfer urine to the acid washed jug in a clean container to avoid burns. Keep on ice or refrigerate. Return it to the lab.

### Using urine pregnancy tests to detect tumors

- *Beta Human Chorionic Gonadotropin* is the substance tested for in urine to confirm female pregnancy or gestational tumors, or gonadal tumors in males. High levels of Beta HCG indicate tumor progression. Low levels indicate effective cancer treatment.
- *Bence Jones proteins* are found in multiple myeloma patients, or when tumors have metastasized to bone. These proteins occur in chronic lymphocytic leukemias and amyloidosis.
- *Urine for Melanin* tests for malignant cutaneous melanomas, and can be done as below, or as a 24-hour urine collection.

*Patient Instructions:* Collect the first morning urine sample, if possible, because it is most concentrated. Clean catch: Wipe the urinary opening front-to-back and do not touch the inside of the container or brush it against the body during collection. At least 50 mL is required. The specimen must not be contaminated with stool, menstrual blood, semen, or prostate extractions. Bring the specimen to the lab immediately or refrigerate. Heat decomposes the proteins, causing false positive results.

### First morning, postprandial, timed, and catheter urine specimens

First morning specimens are most concentrated, so they are preferred for early pregnancy tests, fasting glucose, screening patients for kidney stones (crystals), kidney damage such as glomerulonephritis (protein), and urinary tract infections (nitrate). Postprandial urine specimens are used to monitor diabetics (renal threshold) and should be collected two hours after a meal is finished, when the blood sugar peaks. Timed specimens are for glucose tolerance tests, urobilinogen volume, or quantitative tests for urinary output. Catheter specimens are taken from patients who cannot collect a clean catch but need to be tested for bacterial infection. If the patient is wearing a catheter bag, clamp the catheter for 15 minutes before the collection, wipe off the port with an alcohol swab, and aspirate a sample from the port with a syringe. Remember to unclamp the catheter immediately. If sterile bladder urine or cytology is also required, then the doctor or nurse will draw it from a suprapubic aspiration or catheterize the patient.

### Anuria, oliguria, and polyuria

- *Anuria*: Urinating less than 100 mL in the last 24 hours. This could be because the ureter or bladder is blocked by a stone, or the patient is reacting to a blood transfusion, or the kidney cortex, glomeruli, or tubules are dead.
- *Myoglobinemia*: Hemoglobin from the patient's muscles is excreted in the urine. Cherry red urine, but no RBC's in the microscopic examination. Serum enzymes associated with muscle destruction will be elevated (aldolase, creatine phosphokinase, and lactic dehydrogenase).
- *Oliguria*: Less than 200 mL urinary output in 24 hours. Possibly from: Dehydration, burns, diarrhea and vomiting, excessive sweating (diaphoresis), stones, heart insufficiency, lack of blood flow through the kidneys (renal ischemia), renal disease, or drugs toxic to the kidneys.
- *Polyuria*: Increased urination. If BUN and creatinine are elevated, it could be from obstruction, death of the kidney tubules (necrosis), or diabetic ketoacidosis. If BUN and creatinine are normal, it could be diabetes insipidus or mellitus, brain tumors, or drinking too much water.

### Testing block or fractional urine for S&A

Fractional urine tests a diabetic patient for acetone and glucose to monitor his/her insulin therapy. Perform fractional urines before each meal and at bedtime. Ask the patient to void. Give the patient an 8 oz. glass of water to drink within 5 minutes. Collect a urine specimen. This is a "double-voided" collection. Dipstick or Clinitest and Acetest the urine for glucose and acetone. If the result is negative for both acetone and glucose, then the patient is stable and the insulin therapy is working well. If the patient is "brittle", there will be sharp swings in sugar levels. Use two clean glass test tubes, not plastic, because Clinitest tablets contain sodium hydroxide, and Acetest tablets contain sodium nitroprusside, which heat the tube. Add 10 drops of distilled water and 5 drops of the patient's urine to each tube. Add a Clinitest tablet to one tube and an Acetest tablet to the other. They will boil. Wait 15 seconds and read the results against the color chart.

### Patient falsifying a urine drug test to result in a false-negative:

- *Adding bleach, chromium VI, nitrites, or water* to the urine specimen can produce a false-negative. This is why some drug test urine collections must be witnessed. Watch for a small vial hidden in the patient's underwear or sock. If the patient attempts to adulterate the urine sample with any foreign substance, report it to the doctor.
- Drug levels can be *diluted by drinking 120 ounces of liquid* before the drug test. Cleansing drinks, colonics, diuretics, golden seal, and psyllium also reduce drug levels. If the creatinine level is less than 20 g/dl and the specific gravity is 1.003 or less, inform the doctor that the sample is dilute. Do not accuse the patient of attempting internal dilution, as he/she may have a bona fide problem with ADH the doctor needs to investigate.

- A *prosthetic* belted under the patient's clothes delivers a clean, drug-free urine specimen into the collection container by a straw or through simulated, color-matched genitals connected to a reservoir. This is substitution. Simulated urine can also be used.

## Drugs affecting results

### Drugs that could affect chemical urinalysis results and should be reported on the requisition:

- *Creatinine* can be increased in the urine by nitrofurantoin antimicrobials (Macrobid, nitrofurantoin), anti-Parkinson's medications (Levodopa, Methyldopa), and corticosteroids (Prednisone). Creatinine can be decreased in the urine by thiazide diuretics used to lower blood pressure (Diuril, Naqua, Lozol), androgens, and anabolic steroids like Winstrol to enhance athletic performance.
- *Glucosuria* can be increased by salicylates (Aspirin and aminosalicylic acid), corticosteroids, ephedrine (cold medicine), furosemide (diuretic), and Phenytoin (anticonvulsant). Glucose can be decreased in the urine by old-style mercury diuretics like Mersol, Levodopa (anti-Parkinson's), tetracycline, and Vitamin C.
- *Hematuria* can be increased by anti-inflammatory medicines (Aspirin, acetaminophen, acetophenetidin, and phenylbutazone), anticoagulants (coumarin, Indomethacin), Bacitracin antibiotic ointment, and Amphotericin B antifungal. Proteinuria can be increased by antibiotics (Ampicillin, Bacitracin, cephaloridine, neomycin, penicillin, streptomycin, sulfonamides), anti-inflammatories (Aspirin, aminosalicylic acid), corticosteroids (Prednisone), Chlorpromazine (antipsychotic), thymol (cold medication), and insecticides.

## pH balance

pH balance and what can increase and decrease anion gaps:

- *Anions* are negatively charged ions of chloride and bicarbonate.
- *Cations* are positively charged ions of potassium and sodium.
- *Acidosis* means the body is too acidic and there is insufficient bicarbonate to neutralize the acid. Metabolic acidosis (pH <7.3) is common in diabetics, renal patients, drug overdose, diarrhea, poisoning, and ICU patients with low albumin levels.
- *Alkalosis* means the body is too base from hyperventilation, vomiting, or diuretics. Signs and symptoms of both metabolic acidosis and alkalosis are headache, weakness, twitching, arrhythmia, and coma.
- *Anion Gap (AG)* measures positive and negative ion levels to determine the patient's acid/base balance. The formula is (Sodium + Potassium) - (Chloride + Bicarbonate). Normal AG value is 8—16 mmol/l. Metabolic acidosis is >30 mmol/L. Question results of 20—29 mmol/L. Low AG can be from an incorrect electrolyte test, or lactic acidosis from sepsis. A truly low anion gap can mean multiple myeloma.

## Thin layer chromatography

To use thin layer chromatography to calculate  $R_f$  of a drug screen, line a beaker with filter paper. Put 0.5 cm of eluent in the beaker. Cut a strip of thin layer chromatograph (TLC) sheet to fit inside the beaker. Draw a pencil line 0.5 cm from the bottom of the TLC sheet. Dissolve 1 gram of sample in 100 mL of ethyl acetate, hexane, or methylene chloride and mix. Pipette a drop of sample solution on the TLC sheet just above the pencil line. Place the TLC sheet in the eluent bath inside the beaker, so that only the bottom is covered. Eluent is pulled through the sheet by capillary action. When the eluent reaches about 0.5 cm from the top of the TLC sheet, remove it from the beaker with forceps. Make a pencil line at the highest point where the eluent rose. Air dry. If the test spot is colored, circle it. If the test spot is not colored in daylight, read it under an ultraviolet lamp with goggles and gloves. The sheet fluoresces, but not the blood drop.



# Hematology

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## **Acceptable Chemistry specimen**

Most rejected Chemistry specimens are hemolyzed. Most hemolyzed specimens are collected by non-laboratory personnel. Hemolysis affects analyte concentrations, and especially elevates potassium. If whole blood potassium is elevated, centrifuge it and look at the serum for hemolysis. The specimen must be collected in the correct container (glass or plastic) with the right additive or anticoagulant. There must be sufficient quantity. It must not be clotted if whole blood is needed, or contaminated with other additives because the wrong order of draw was used during collection. It must be delivered to the lab within the testing time limit, and at the correct temperature for the requested test. The label must be in indelible ink and include the patient's name, identification number, tests, requisitioning doctor's name, collection date and time. The accompanying requisition is most useful if it includes a tentative diagnosis and drug list to guide the testing technician who finds anomalies.

## **Cold agglutinins**

Cold agglutinins are antibodies made by the immune system to fight disease. The normal value is 1:40. Cold agglutinins make red blood cells adhere to each other in a Rouleaux formation when they are chilled. RBC clumps can clog Coulter Counters and other automated lab equipment. Cirrhosis of the liver, congenital syphilis, cytomegalovirus, Hepatitis C, lymphoma, malaria, mononucleosis, mycoplasma pneumonia, myeloma, old age, rheumatoid arthritis, and scleroderma can all raise the level of cold agglutinins. Cold agglutinins are tested a week after pneumonia symptoms develop to rule out mycoplasma infection while waiting for culture and sensitivity results. However, taking antibiotics invalidates the test. Rarely, cold agglutinins can cause hemolytic anemia. Raynaud's phenomenon patients have high cold agglutinin titers, and must avoid becoming chilled, because they will experience frostbite-like symptoms – white, waxy ears, fingers, nose and toes, followed by numbness, burning pain, and thrombosis.

## **False readings and results**

### Pseudohyperkalemia, hyperammonemia, and falsely decreased bilirubin

Pseudohyperkalemia is a false reading of increased serum potassium. It can be caused by poor venipuncture technique or transfusing hemolyzed blood. Hyperammonemia is elevated ammonia level in the blood, affecting pH. Tightening the tourniquet too much during venipuncture, storing the tube too long before testing, or hemolyzing the sample cause the ammonia level to be artificially high. The false result may give the impression the patient has a serious digestive disorder. Mark on the requisition anything else that would elevate the test: Smoking, not fasting for at least 8 hours before collection, or heavy exercise. The CLT can cause an artificially lowered bilirubin count by leaving a specimen

tube exposed to light at room temperature for one hour; shaking the specimen or allowing air bubbles in it; not warning the patient to avoid antibiotics, antimalarials, barbiturates, diuretics, fasting more than 8 hours, fatty meals, orange vegetables (carrots and yams), salicylates, and steroids before the test.

#### Instances which cause a false result:

- *Drawing blood from an arm with an IV:* Dextrose IV can lower the serum amylase level.
- *Allowing bubbles in a specimen tube:* Bubbles in an arterial blood gas syringe can increase the gas levels; bubbles in a venous bilirubin sample can decrease the bili level.
- *Not icing a sample immediately:* Blood gases are untestable if they are kept at room temperature, even for a few minutes, because ice slows down cellular respiration.
- *Using the wrong anticoagulant:* Using any anticoagulant other than oxalate in a grey stoppered tube causes an artificially low blood sugar, because the cells continue to eat glucose in the tube.
- *Scheduling an examination before a blood test:* Scheduling a prostate exam or TURP procedure less than one week before a PSA test can artificially increase the PSA to indicate prostate cancer.
- *Failing to ask a patient if he/she has pre-existing conditions before a test:* Failing to ask a patient if she has had an amniocentesis, a history of cirrhosis, or hepatitis could result in misdiagnosis from an artificially high AFP level. Failing to ask a patient if she is pregnant could invalidate a Beta-HCG level.
- *Mislabeling a sample:* Mislabeling a semen sample could lead to an incorrect diagnosis of infertility.

### **Using a Unopette**

When using a Unopette to fill a Neubauer-ruled hemocytometer to manually count white blood cells, select a Unopette for white blood cells. Push a capillary pipette, still sheathed in its shield, through a Unopette reservoir's neck to open it. Disconnect pipette from shield with a gentle twist. Hold the pipette to a punctured finger, or inside an EDTA or citrated blood tube to fill it. Insert the filled pipette into the reservoir. Squeeze the reservoir. Seal the open pipette's top with your gloved finger. Release pressure on the reservoir and pipette opening. The blood is drawn into the reservoir filled with diluent. Squeeze the reservoir to mix the cell suspension in the Unopette. Pipette 10 microliters into the hemocytometer chamber. Place a slide cover on top to prevent evaporation. Wait 10 minutes for cells to settle. Count the cells in nine large squares at 10x magnification. Report the total WBC count per cubic millimeter as the  $(\text{number of cells counted} \times 10 \times 100) \div 9$ .

### **Automated versus manual smears**

Large labs use slide-makers, (e.g., Beckman Coulter Gen-S) to smear, stain, and label 12 slides simultaneously. Automation saves \$40,000 annually on labor and reduces exposure

to infectious blood. In a small lab, or when hematology results are questioned, follow this procedure: Polish two slides with lens paper to prevent streaks and bubbles. Fill a blue-ringed, non-heparinized capillary tube with EDTA or citrated blood. Hold the capillary tube vertically over the unfrosted end of one glass slide. Let one blood drop fall ½" from the slide end to form the thickest end of the smear. Hold the second slide at a 35° angle over the blood drop. Touch the blood drop with the second slide, until the blood spreads to within 1/8" from the edge. Push evenly and gently with the spreader to smear blood the length of the slide. Near the end, lift the spreader gradually to make a round, feathered, thin end. Hesitating causes vertical bars; spreading too slowly causes long, thin smears; spreading too quickly causes short, thick smears.

### **Romanowsky's stain**

Romanowsky's stain is composed of eosin Y, oxidated methylene blue, and methanol. Use it to see WBCs and malaria parasites. More stable variants of Romanowsky's are: Giemsa, Jenner's, Leishmans, and Wright's. Mix 20 mL of Giemsa stain with 240 mL of distilled water. Place a dry, smeared slide in a staining rack. Pipette Wright's stain mixture over the slide to a depth of 1/8". Wait 2 minutes. Pipette 1/8" of the Giemsa mixture over the slide. Blow on it to mix the Wright's and Giemsa stains together. It will look metallic when mixed correctly. Wait 4 minutes. Wash the slide with distilled water from a squeeze bottle for 30 seconds. Air dry before reading. The buffer must be 6.4—6.7. Too much acid will make it pink and the nucleus will be unclear. Too much alkali will blacken cells and make structures indistinct.

### **CSF collection**

Cerebrospinal fluid (CSF) is collected *by an M.D.* from a lumbar puncture. Assist the doctor by preparing a collection tray containing: Iodine prep; alcohol prep; 3 cc of 1% lidocaine; 25g, 5/8" needle; 22g, 1.5" needle; atraumatic spinal needle (to prevent post collection headache); syringe; four sterile red stoppered tubes; 4X4 gauze; sponge forceps; sterile towels; small basin; and a Band-Aid. Wash your hands, mask, gown, and glove. Explain to the patient in general terms what you and the doctor are going to do. Position the patient on his/her side, curled up chin to chest, and knees to abdomen. If the doctor is right handed, the patient is left lateral. If the doctor is left-handed, the patient is right lateral. The doctor anesthetizes the area, inserts the needle between L3—L4, and collects the fluid. Label one tube each for cell count, glucose and protein, gram stain and culture, virology/mycology/cytology. You may need an extra tube if globulin immunoelectrophoresis is ordered. Bandage the puncture.

### Normal values for CSF

Normal cerebrospinal fluid is clear and colorless. Hold the tubes of CSF against a white piece of paper backlit by a bright white light. Record any bloody streaks, turbidity, or discoloration. Xanthochromia is yellowed CSF, perhaps indicating: A traumatic tap; Herpes Simplex Virus encephalitis; increased protein; jaundice; or subarachnoid hemorrhage (SAH) between 12 hours and 2 weeks old. RBC count should be 0. Turbid CSF indicates

meningitis and increased WBC count, which should be less than 5. Neutrophilia indicates bacterial meningitis and lymphocytophilia indicates viral meningitis. Protein above 45 mg/dl indicates meningitis, immunoglobulin production from multiple sclerosis, or tumor blocking the flow of CSF. Glucose below 50 mg/100 mL indicates bacterial meningitis. Normal values are: Chloride 700—750 mg/dl, glutamine 6—15 mg/dl, LDH 2.0—7.2 U/mL. You should see no fungus or malignant cells on a slide. The culture should have no growth.

## Quality assurance problems

Five quality assurance problems that could adversely affect hematology tests:

- Fill lavender stoppered tubes to capacity, or too much EDTA causes the RBC's to shrink and affects the hematocrit. RBC and WBC counts and hemoglobin are unaffected.
- Wright's stain with a buffer that has a pH of less than 6.4 causes RBC's to suck up eosin red dye. RBC's on the smear look too pink and WBC's show poor detail in the nucleus.
- An unbalanced centrifuge set for the wrong number of rotations traps plasma in the microhematocrit tube and produces hematocrit errors. Balance and set microhematocrit centrifuges at 12,000 g for 5 minutes.
- Patients at high altitudes have higher hemoglobin, hematocrit, and RBC counts than patients at sea level, to compensate for the lower oxygen content of the air.
- Automated differentials on cell counters can identify too many monocytes when the patient has abnormal lymphocytes, chronic myelomonocytic leukemia, inflammation, or myeloproliferative disease. If the cell counter sees 15% monocytes, disregard it, make a peripheral smear and perform a manual count.

## Preference of manual cell count

The reason you would do a manual cell count on a peripheral blood smear instead of using a cell counter (e.g., Cellavision Diffmaster) is that CAP requires manual quality control RBC, WBC, and platelet counts every eight hours. Automated cell counts flagged abnormal also trigger manual reviews. Patients' blood may be normal, but the quality of the stain used was poor (too acid or alkali), the specimen was diluted incorrectly, or the machine calculation was inaccurate. Run a commercial control with known values through the cell counter. Cell counters overcount monocytes when there are many immature granulocytes. If the monocyte count is >15%, differentiate 100 cells manually to rule out chronic myelomonocytic leukemia, inflammation, or abnormal lymphocytes. Clotting at room temperature causes cell counters to falsely read low hematocrit and RBC count, and high MCV, MCH, and MCHC indices. Automated cell counters count all nucleated cells, including immature RBCs. If you see RBCs with nuclei in the peripheral smear, correct the falsely elevated WBC in the automated cell count. Find the number of nucleated RBCs for every 100 WBCs. The correction formula is:  $WBC \text{ Count} \times 100 / 100 + \text{number of nucleated RBCs}$ .

## Thrombocytes

Platelets are also called thrombocytes. They are blood clotters, so a platelet count is used to diagnose prolonged bleeding, bone marrow cancer, or unexplained bruising. Platelets grow from stem cells (megakaryocytes) in bone marrow. Kidneys and liver produce thrombopoietin to make bone marrow produce more thrombocytes. Normally, adults have 150,000 to 450,000 platelets per microliter of blood. Babies have 200,000 to 475,000. Increased platelet count is thrombocytosis, which is seen in myoproliferative disorder and thromboembolisms. Decreased platelet count is thrombocytopenia. Platelet levels in the 50,000 to 20,000 per microliter range indicate uncontrollable bleeding may occur. Thrombocytopenia occurs in patients with leukemia, chronic stomach ulcers, and Idiopathic Thrombocytopenia Purpura (ITP).

## Hematocrit tests

A hematocrit (Hct) test separates the blood cells from the plasma in a centrifuge as part of a complete blood count (CBC). Hct indirectly measures red blood cell mass, so if the RBCs are of normal size, then the Hct should confirm the RBC count. Patients with macrocytic, microcytic, or iron deficiency anemia with small RBCs will not have parallel Hct and RBC counts. Results are reported as Packed Cell Volume (PCV), meaning the percentage by volume of packed red blood cells in whole blood. Normal values for venous blood are: Males 42% to 52%; females 36% to 48%. Microhematocrit readings from capillary tubes are a little higher. Babies have higher hematocrits than adults because they have more macrocytic RBC's. An abnormal hematocrit suggests follow-up tests must be done for a firm diagnosis. Low hematocrit readings (less than 30%) may indicate many diseases: Adrenal insufficiency, anemia, burns, Hodgkin's disease, leukemia, or poisoning. High hematocrit can be from erythrocytosis, polycythemia vera, or shock.

## Hemoglobinometers

Test the hemoglobinometer with known controls first. Then, polish a clean hemocytometer slide with lens paper. Fill a blue-ringed, unheparinized capillary tube with blood. Place one drop of blood on the hemocytometer slide. Roll the heparinized, wooden hemolysis stick over the blood drop until the blood is hemolyzed and transparent (about 30 seconds). Place the cover slip over the slide. Slide them together into the hemoglobinometer. Look through the viewer. Adjust the light until the two fields you see are exactly the same color. (Most hemoglobinometers use green and black.) Read the scale (usually on the side of the meter) and record the hemoglobin level. (Normal values are 12—16 g/dl for women, and 14—16 g/dl for men.) Disassemble the slide and cover slip. Sanitize, disinfect, polish, and case them. Wipe the test area and outside of the hemoglobinometer with disinfectant. Hemoglobinometer readings with capillary blood show about 10% false-positives for anemia, as compared to venous Coulter Counter readings. Hemoglobinometers are for point-of-care testing for “ballpark” estimate readings.

## RBC indices

Three RBC indices are used to diagnose anemia. They are: Mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); and mean corpuscular volume (MCV). “Normal Cell Indices” means the blood smear shows normally colored and shaped cells that are not anemic.

MCH measures the amount of hemoglobin per cell. 27—30 picograms per cell is normal. MCHC measures the amount of hemoglobin relative to the size of the cell (hemoglobin concentration per cell). 32—36 grams per deciliter is normal. More than 36 g/dL usually means spherocytes are present. If the RBC’s are pale (hypochromic), the MCHC will be lower than 32 g/dL. MCV measures the average size of RBC’s. 88—100 femtoliter is normal.

Hemoglobin, hematocrit, and RBC count are used to calculate MCH and MCHC. To calculate MCH, divide the hemoglobin result (Hgb) by the red blood cell count (RBC). To calculate MCHC, divide the hemoglobin result (Hgb) by the hematocrit result (Hct.). MCV is a calculated independently.

## ESR test

An erythrocyte sedimentation rate (ESR or sed rate) measures how far blood cells with anticoagulant (EDTA or sodium citrate) will fall in a clump (aggregate) in a Westergren or Wintrobe tube in one hour due to changes in plasma proteins (Rouleaux formation). Normal RBC’s do not form Rouleaux, and they settle slowly. Normal values are: Males 0—15 millimeters per hour; females 0—20 mm/h; children 0—10 mm/h. RBC settling for patients over 50 years old may normally measure 5—10 mm/h more. If RBC’s settle quickly, the Rouleaux indicates some type of inflammation, necrosis, or parasites are present and further tests are required, but a sed rate doesn’t definitively diagnose a disease. A high ESR can indicate many diseases: anemia, arthritis, cancer, heart attack, lupus, pelvic inflammatory disease, kidney disease, pneumonia, poisoning with heavy metals, syphilis, thyroid disease, toxemia, tuberculosis. A low sed rate can be from heavy blood loss.

## Drug interference

Drugs that could interfere with Coombs’ test and erythrocyte sedimentation rate in a hematology lab:

- *Coombs’ test* (direct and indirect antiglobulin test) for anemia can be false-positive if the patient takes these drugs, and they should be written on the requisition: Chlorpromazine tranquilizer; Chlorpropamide antiglycemic; Ethosuximide or Phenytoin anticonvulsants; Hydralazine antihypertensive; Isoniazid antitubercular; Levodopa anti-Parkinson’s medication; Mefenamic acid or phenylbutazone pain killers; Melphalan chemotherapy to treat ovarian or muscle cancer; Procainamide

and Quinidine antiarrhythmia medications; Quinine antimalarial; Streptomycin, sulfas, and tetracycline antibiotics.

- *Erythrocyte sedimentation rate (ESR)* to monitor inflammation can be *increased* if the patient takes these drugs, and they should be written on the requisition: Dextran anticoagulant; Methyldopa anti-Parkinson's medication; Methysergide for migraine headaches; Penicillamine immunosuppressant to treat rheumatoid arthritis; Theophylline to treat asthma; Trifluoperidol antipsychotic; Vitamin A. ESR can *decrease* if the patient takes these drugs, and they should be written on the requisition: Quinine; salicylates (Aspirin); steroids (cortisone).

## Reticulocyte count

Counting retics indicates how fast bone marrow is producing blood. Use methylene blue stain to see the RBC reticulum properly. Use a Miller disc to manually count red blood cells on the slide. Place the Miller disc on the ocular to divide the field into two squares, one of which is nine times larger than the other. Count reticulocytes only in the large square, and RBC's only in the small square. Look for polychromasia on the blood smear — blue or grey-tinged, immature RBCs (retics) in which RNA is still present. Polychromic cells have been circulating in the bloodstream less than two days, because that is when RBCs lose their RNA and look red on a routine smear. Correct the retic count for anemic patients because the percentage of retics is not a true measure of production:  $\% \text{ retics} \times \text{Hct} / 45 = \text{corrected reticulocyte count}$ .

## White blood cells

The white blood cells:

- *Basophils*: Black or purple. Granulocytes. 1%. *Increase*: Asthma, chronic myelocytic leukemia, Crohn's disease, dermatitis, estrogen, hemolytic anemia, Hodgkin's disease, hypothyroidism, polycythemia vera, and viruses. *Decrease*: Allergies, corticosteroids, hyperthyroidism, pregnancy, and stress.
- *Eosinophils*: Orange-red. Granulocytes. 1%—3%. Double-lobed nucleus. *Increase*: Allergy, asthma, or parasitic infestation. *Decrease*: Cushing's disease or glucocorticoids use.
- *Lymphocytes*: Dark, large nucleus surrounded by thin cytoplasm rim. Agranulocytes. 15%—40%. *Increase*: Antigens or chronic irritation. *Decrease*: AIDS. Make proteins for immunoglobulins and cytokine production. Small B and T cells secrete antibodies and regulate the immune system. Large Natural Killer cells lyse tumors and virus-infected cells. Children 4 months—4 years normally have inverted differential (relative neutropenia and increased lymphocytes).

- *Monocytes:* Lavender. Agranulocytes. 2%—8%. *Increase:* Myeloproliferative process, like an inflammatory response or chronic myelomonocytic leukemia (CMML). *Decrease:* Hairy cell leukemia.
- *Neutrophils:* Pink cytoplasm, dark nucleus. Granulocytes. 50%—70%. *Increase:* Burns, kidney failure, heart attack, cancer, hemolytic anemia. *Decrease:* Leukemia and abscess. Phagocytic. Segmented nucleus (seg) is a mature cell. Banded nucleus (band) is immature.

## **Leukemia**

Leukemia is blood cancer that occurs when bone marrow produces abnormal white blood cells that crowd out normal cells. Usually, it results from: Working with chemicals, especially benzene and formaldehyde; radiation exposure; chemotherapy; Human T-cell Leukemia Virus (HTLV-1); myelodysplastic syndrome; and damaged chromosomes (for example, in Down's Syndrome). The four common types of leukemia in order of prevalence are: acute myeloid (AML) that affects all ages; chronic lymphocytic (CLL) that affects mostly people over 55 years old; chronic myeloid (CML) that affects mostly adults; and acute lymphocytic (ALL) that affects mostly children and few adults. Leukemia patients get frequent infections because, although they have many WBC's, these are too immature to effectively fight infection. Leukemia gives patients bone pain, swollen spleen and lymph nodes, fever, bruising, bleeding, headaches, and weakness.

## **Coulter Counter**

Turn on the Coulter Counter 10 minutes before testing to build up vacuum suction. Mix 0.2 mL of blood cell suspension with 10 mL of Tris solution. Open the glass valve to vertical to load the sample into the counter. When you see a horizontal green line on the monitor screen, turn the valve horizontally to begin counting for around 13 seconds. You will see vertical marks on the monitor when a cell or artifact is counted. The numbered count of cells in 0.5 mL appears on the register. Multiply the number of cells by 100 to get the cell count per milliliter and record it on the patient's chart. Flush the counter by placing the electrode into a vial connected to 100 mL of Isoterge/Tris solution. Turn the right valve (A) vertically. Turn the left valve (B) vertically. Wait 10 seconds. Turn the left valve (B) and then the right valve (A) horizontally. Leave the electrode in the Isoterge/Tris solution. Turn the counter off.

### Troubleshooting Coulter Counter results

Vertical marks on the monitor might be artifacts or microclots instead of cells. If the Coulter Counter keeps counting longer than the standard 13 seconds, then check the sample loading aperture for clogs. If you find a debris clog, then brush it away and flush the counter with Isoterge/Tris solution. Retest the sample. If the sample count is less than 3,000 or more than 99,900 per 0.5 mL, then you probably did not mix the cells sufficiently with Tris solution. If the solution is too concentrated, multiple cells pass by the aperture together, but are counted as only one cell. Check the EDTA tube for clots, make another



dilution, and retest the sample. If it is still out of range, count manually or try a citrated tube for the same patient.

### **PTT and aPTT assays**

Use a blue citrated tube filled to  $\geq 90\%$  capacity (5 mL). Keep at room temperature. For patients on heparin, collect blood one hour before the injection, and test within one hour. Do not draw blood from a heparin lock arm. For heparin-free patients, test blood within 24 hours of collection. If the tube is clotted, hemolyzed, mislabeled, in the wrong color tube, or thawed out in transit, do not proceed. Notify the phlebotomist to redraw the sample. Invert the tube 6 times before testing to mix blood with citrate. Add 2 mL of blood to an ACT test tube, shake well, and load the sample into a Hemochron test well for automated testing. Listen for the beep. Record the results. (If manual PT, mix two parts platelet poor plasma with one part phospholipid/tissue factor. For manual aPTT, substitute phospholipid/negatively charged surface active agent. Mix with calcium.) If delayed beyond 24 hours, centrifuge at 1,500g for 15 minutes. Pour platelet poor plasma into a purple Transpak tube. Freeze it until testing.

### **INR, PT and PTT**

INR (**international normalized ratio**) checks clotting Factor VII to I. It standardizes different tissue factors (thromboplastin) used worldwide for testing. Normal INR is 0.9—1.2. If a patient has an INR under 2.0, there is little bleeding. If a patient has an INR of 3.0—4.5, there is heavy bleeding (hemorrhage). Most patients on anticoagulant therapy (Coumadin, heparin, warfarin) are kept with an INR of 2.0 to 3.0, depending on their condition. If the patient is very likely to develop blood clots, the doctor may push the INR to 3.5. Therapeutic INR is checked every 4—6 weeks. Normal PT is 10—12 seconds. Therapeutic value is 13—18 seconds. PT checks Factor VII to I. Normal PTT is 25—38 seconds. Therapeutic value is 38—76 seconds. PTT checks Factor XII to I. PT and PTT are being phased out in favor of INR. Prolonged INR may be due to bile deficiency, cirrhosis of the liver, lack of Vitamin K, or small intestine disease.

### **Clotting time test**

The doctor orders clotting time test when the patient has a bleeding disorder, or before major surgery. It measures the time a surgical incision takes to clot, the blood's ability to clot, and estimates the time to close off a wound. Gather together: A clock or watch with a second hand; 1 sterile glass tube; 2 isopropyl alcohol swabs; cotton balls; 1 sterile razor. Cover the patient's clothing with paper towels. Swab the forearm with alcohol. Allow to dry. When the second hand reaches 12:00, incise the site. Collect 5 mL of blood in the glass tube and place in a rack. Start timing. Apply pressure over the site with cotton to absorb blood. Check every thirty seconds until bleeding stops. The tube should clot in 5—15 minutes and retract in 30—60 minutes. Record the tube and forearm results on the requisition. Prolonged clotting time means coagulation protein deficiency.

Hypofibrinogenemia is a poorly formed, friable clot. Enhanced fibrinolysis means the clot retracts too early.

#### Clotting process:

- *Stage I:* An injury occurs, and for the first three to five minutes afterwards the platelets are mobilized, called Phase I. Most coagulation disorders are Phase I problems. In Phase II, tissue thromboplastin forms in response to the distress call (Factor III) from the injured site. You need all of these to form tissue thromboplastin: Calcium and factor V, VIII, IX, X, XI, and XII. (Hemophilia, an inherited clotting disorder of males passed along the female line, is a deficiency of Factor VIII.)
- *Stage II:* Fibrinogen changes to fibrin and prothrombin Factor II changes to thrombin in the next eight to 15 seconds. You need Factor II, V, VII, and X for Stage II to work.
- *Stage III:* In the next second, thrombin and fibrinogen Factor I form a clot and then Factor XIII secures the clot.
- *Stage IV:* Clot formation ceases once the injury is plugged, called fibrinolysis, when plasminogen changes to plasmin.

#### **Clotting factors**

The clotting factors:

- *Factor I:* Fibrinogen makes clots to close wounds.
- *Factor II:* Prothrombin requires Vitamin K; interacts with thrombokinase and calcium to form thrombin.
- *Factor III:* Thromboplastin (tissue factor) accelerates clotting.
- *Factor IV:* Ionized calcium (Ca<sup>++</sup>); free calcium circulating in blood.
- *Factor V:* Proaccelerin (labile factor); destroyed by heat; speeds conversion of prothrombin to thrombin.
- *Factor VI:* Now unassigned, formerly accelerin.
- *Factor VII:* Proconvertin (stable factor) requires Vitamin K; heat stable; thromboplastin makes it form Factor VIIa, which causes Factor X to form Xa.
- *Factor VIII:* Antihemophilic factor A (AHF) or Antihemophilic globulin (AHG), lack causes Hemophilia A.
- *Factor IX:* Antihemophilic factor B (Christmas factor) requires Vitamin K; storage stable; acts with Factor VIII, calcium and platelet factor 3 to activate Factor X; lack causes Hemophilia B.
- *Factor X:* Stuart-Prower factor requires Vitamin K; glycoprotein; storage stable.
- *Factor XI:* Plasma thromboplastin antecedent; storage stable; lack causes Hemophilia C
- *Factor XII:* Hageman (glass factor); storage stable; activated by torn vessels.
- *Factor XIII:* Laki-Lorand factor (fibrin stabilizing factor); glycoprotein activated by thrombin and calcium

## Prothrombin times

Prothrombin times can be increased by many common drugs. These include: Pain killers (Acetaminophen, Indomethacin, Mefenamic acid, salicylates); antibiotics (Chloramphenicol, Erythromycin, Neomycin, Streptomycin, Sulfas); anticoagulants (Coumadin, heparin, warfarin); antidepressants (MAO inhibitors); blood pressure reducers (methyldopa and diuretics to treat edema); blood sugar regulators (glucagon and tolbutamide); quinines and quinidines to treat malaria and regulate the heartbeat; thyroid medications. Your elderly patients may take several of these medications. These drugs work two ways to increase the time it takes the blood to clot:

- They push anticoagulants away from their bonds with plasma proteins, making more anticoagulants circulate in the blood.
- They diminish friendly bacteria in the gut, which curtails Vitamin K production.

Apply direct pressure to the site with plenty of clean cotton. Wait a few minutes before allowing the patient to leave, as excessive bleeding will cause a hematoma to form.

*Example:* The patient is prone to developing blood clots. His prothrombin time has been stabilized with an anticoagulant and he takes additional drugs. The kinds of drugs that could critically decrease his prothrombin time if they were removed abruptly from the treatment regimen: Some drugs cause the liver to make enzymes to metabolize anticoagulants (coumarin and phenindione) and decrease the prothrombin time. If the patient suddenly stops taking the additional drugs, the prothrombin time may drop so much that there is a danger of a thromboembolism (traveling blood clot) forming. Drugs that could decrease the prothrombin time include: Antacids (Tums, Pepcid); antihistamines (allergy medicines); anti-inflammatories for gout (colchicine); barbiturates (heptabarbital, phenobarbital, secobarbital); birth control pills; griseofulvin (fungal infections like athlete's foot, jock itch, and ringworm); sleeping sedatives (chloral hydrate, ethchlorvynol, glutethimide); steroids (anabolics, cortisone, corticosteroids); Tetracycline; Vitamin C; Vitamin K. If the patient is taking any of these regularly with the anticoagulant, the doctor will have to wean him from the drug slowly to prevent the reaction of clot formation.

## Erythrocytes

Erythrocytes (RBC's) are red blood cells that grow from stem cells (erythroblasts) in bone marrow. RBC's carry oxygen to cells and carbon dioxide away from cells. Iron, protein, Vitamin B12 and folic acid determine RBC growth. Kidneys produce erythropoietin to make bone marrow produce more erythrocytes. RBC's are barbell-shaped ("bi-concave discs") of about 7 micrometers across at maturity. An immature red blood cell (reticulocyte) has a dark nucleus, but a mature RBC has no nucleus. A mature, normal RBC has "central pallor" where its nucleus once existed, and that pale area helps it transport oxygen efficiently. RBC's live around 120 days and then are destroyed, mainly by the spleen. Old red blood cells look more spherical than barbell-shaped, and that shape change makes them fragile. New RBC's are flexible so they can squeeze through capillaries. Babies

have a very high RBC count (4.8 to 7.2 million per cubic millimeter of blood). Adult males have 4 to 6 million, and adult females 4 to 5 million. Pregnancy lowers RBC counts.

The following are important terms regarding RBSs:

- *Anisocytosis*: Unequally sized RBCs.
- *Hypochromia*: Pale RBCs from lack of hemoglobin (anemia).
- *Leptocyte*: A thin, flat RBC (codocyte, target cell or Mexican Hat cell) with a dark centre surrounded by a clear ring, and a dark edge. Common in sickle cell anemia and thalassemia. Leptocytes don't burst easily in the osmotic fragility test because they have more surface area: volume (left curve shift).
- *Phagocytosis*: Monocytes in the blood and macrophages in the bone marrow, liver, lungs, and spleen surround, ingest, and destroy intruders with enzymes
- *Poikilocytosis*: Irregularly shaped RBCs.
- *Neutropenia*: Reduced neutrophils, usually from infection or bone marrow suppression.
- *Osmotic Fragility*: Detects RBCs that are more fragile or resistant than normal by making them absorb hypotonic saline until they burst (hemolysis).
- *Spherocyte*: Small, round RBCs with no central pallor. Spherocytes burst easily in the osmotic fragility test because they have less surface area: volume (right curve shift).
- *Heinz bodies* are many purple inclusions on the RBC cell membrane that indicate hemolytic anemia. They are decomposed hemoglobin that is normally removed from the cell by the spleen. Supravital stain is needed to see Heinz bodies.
- *Howell-Jolly bodies* are usually single dots on the RBC. The dot is a remnant of the nucleus when it is expelled as the RBC leaves the bone marrow, or if the nucleus ruptured (karyorrhexis). Jolly bodies are seen in the smears of newborn patients, or adults with diseases of the spleen, or hemolytic and megaloblastic anemias. Use Wright or Giesma stain to see Jolly bodies.
- *Basophilic stippling* is many blue dots found in the RBC's of patients with alcoholism, bacterial infections, hemolytic or megaloblastic anemia, lead poisoning, and thalassemia. Brilliant cresyl blue stain is needed to see stippling.
- *RDW*: Red blood cell distribution width, a parameter used by a Coulter Counter machine to adjust for variation in RBC size. It correlates with anisocytosis seen on the blood smear. Normal range is 11.5% — 14.5%.
- *Deoxyhemoglobin*: Hemoglobin that off-loaded oxygen and took on carbon dioxide, causing purplish RBCs.
- *Hemoglobin electrophoresis*: Differentiates hemoglobin into HbA and HbA2 (normal); HbS (sickle cell); HbC (hemolytic anemia); Hb F (fetal/infant).
- *Hemopoiesis*: Blood formation. In embryos, blood forms in the liver and spleen 20 weeks. After 20 weeks, bone marrow takes over blood formation. Red marrow makes most blood cells at the ends of long bones, vertebrae, ribs, pelvis and skull. Lymphocytes are produced in the thymus, spleen, and lymph nodes. Adult livers form blood in case of stress or trauma.
- *Hyperchromic*: Too dark or pigmented. Hyperchromic anemia has increased hemoglobin weight compared to RBC volume.

- *Macrocytic*: Abnormally large cell. Large red blood cells lack folic acid and signal anemia.
- *Microcytic*: Tiny cell. Cell division in bone marrow is prolonged due to lack of hemoglobin from iron deficiency, sickle cell anemia or thalassemia.
- *Normochromic*: Normally colored red blood cell, indicating no anemia is present.
- *Oxyhemoglobin*: Normal hemoglobin carrying oxygen (ferrous or 2+) in scarlet red cells.

The following are common RBC disorders:

- *Curve shifts*: Acidosis and spherocytes cause increased oxygen release and the oxyhemoglobin dissociation curve shifts right. Alkalosis, blood transfusions, Hb Kansas, and leptocytes cause the curve to shift left.
- *Hemosiderin*: Pathological ferric (3+) ions in RBC's (siderocytes), usually from a hemorrhage. Prussian blue stain is required to see hemosiderin.
- *Sickle cell anemia*: HbS is a genetic disorder of African peoples where the RBC's bend and stick in the capillaries, causing great pain. Sickled RBC's only live 10 — 20 days. It might be an over-adaptation to malaria parasites.
- *Thalassemia*: A group of genetic anemia disorders. Alpha affects India and southern China. Beta affects Mediterranean people (Italians, Greeks, Arabs, and North Africans). Hb H disease causes deformed bones, enlarged spleen, and fatigue. Cooley's anemia is lethal if not treated with blood transfusions and iron chelation.

## Differentiating leukemias

The common types of stains used to differentiate leukemias in a morphological examination, and some diseases they are used to diagnose:

- *Leukocyte Alkaline Phosphatase*: LAP stain highlights neutrophils. Use to differentiate between high numbers of WBC's that are not leukemia (leukemoid reaction) from chronic granulocytic leukemia. Normal values are 40 to 100 LAP units, but can also be present in hemolytic or iron deficiency anemia, mononucleosis, and hepatitis. Low values of 0 to 15 LAP units indicate CML, idiopathic thrombocytopenia purpura, siderocytic anemia, or muscular dystrophy. Increased LAP indicates chronic granulocytic leukemia, polycythemia vera, hairy cell leukemia, Hodgkin's disease, and multiple myeloma.
- *Periodic Acid-Schiff*: A cytochemical stain. Use PAS stain to diagnose acute lymphoblastic leukemia (ALL). Mature granulocytes absorb PAS, but mature RBC's do not. If erythroblasts absorb PAS conspicuously, it suggests M6 erythroleukemia.
- *Sudan Black B*: Use SBB for acute leukemia patients, to differentiate between immature cells. SBB stains lipids (fats) in myeloid leukemia that are absent in lymphoid leukemia.

## Dangerous hemoglobin derivatives

Three dangerous hemoglobin derivatives that prevent oxygen from moving around the body adequately:

- *Carboxyhemoglobin*: Hemoglobin bound to carbon monoxide instead of the usual oxygen. Carbon monoxide has a greater binding capacity (affinity) to hemoglobin than oxygen does, so RBC's absorb carbon monoxide more readily. Normally, 3% of RBC's carry carboxyhemoglobin, but heavy smokers may have 15%. Over 50% carboxyhemoglobin concentration is carbon monoxide poisoning, signified by a cherry red face.
- *Methemoglobin*: The iron molecule is ferric 3+, so cannot carry oxygen or carbon dioxide. The dissociation curve (OD) shifts left. Patients may have a genetic lack of G6PD enzyme, or chemical exposure (anesthetics, sulfonamides, quinones, paints or inks). Arterial blood turns chocolate brown. Cyanosis results at 10 — 15% methemoglobin levels. Headache and shortness of breath develop at 35% — 40%. Stupor results at 60% concentration. Methemoglobin concentration over 70% is lethal. The antidote for methemoglobin poisoning is IV and oral methylene blue.
- *Sulfhemoglobin*: It occurs when a patient takes sulfonamides or phenacetins. No sulfhemoglobin is normally present. If levels reach 10 g/dL, the patient will be cyanotic (blue), but undamaged.

## Anemia

Anemia means “lack of blood”. Body cells are oxygen-deprived (hypoxia) when there are too few red blood cells to feed them oxygen, or if the RBC's are poorly shaped, or lack enough hemoglobin to transport the oxygen. The body tries to make the RBC's it has more efficient, so it increases the 2,3-DPG that allows hemoglobin to take on oxygen. This changes the oxygen dissociation curve (OD). Hemorrhagic anemia from trauma or tumors is an example of too few circulating RBC's. Iron deficiency anemia (IDA) can be from poor diet, chronic bleeding (ulcers, heavy menstruation), or pregnancy. Pernicious anemia is from lack of Vitamin B12 or Intrinsic Factor in the stomach to process it for RBC production. Folate deficiency anemia is from the lack of folic acid in the diet or during pregnancy. Genetic anemias include Sickle Cell Anemia, Fanconi Anemia, and Thalassemia. Aplastic anemia is when the bone marrow stops making enough blood cells, for example, in cancer patients, after radiation or chemical exposure, lupus, rheumatoid arthritis, and viral diseases like HIV.

## GSA

The GSA (General Semen Analysis) kit checks sperm count, motility, vitality, and white blood cell count in the ejaculate. A flow cytometer can check for 100 times more cells than a technician can manually, using a lighted microscope with a counting chamber. Using a GSA kit for semen analysis gives the lab better quality control because the cell count is more precise and the technician's skill and experience are less critical. It is easier to repeat

the test with a flow cytometer than it is manually. The standard deviation between testing technicians is less with a flow cytometer than it is with the manual Routine Method.

- A high white blood cell count can cause background staining and a false result. The patient must wait until his infection is cured, and repeat the semen collection.
- A fungus infection can cloud the semen and cause auto-fluorescence, making it difficult to read accurately.
- If the semen is very thick, it may need to be diluted with Bromolin so that it can be read.

# Immunohematology

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## Criteria blood donors

Accept	Defer	Exclude
Temperature < 37.5°C	Aspirin-free (36 hrs.)	Babesiosis
		Blood exposure
Weight > 110 pounds	No oral polio or oral typhoid vaccine (2 wks.)	Chagas disease
		Creutzfeldt-Jakob risk in self or family
Heart rate 50—100 bpm	No live attenuated injected vaccine for mumps, rubeola	Hepatitis B or C in self or household
Blood pressure <180/100	No rubella or varicella vaccines (4 wks.)	HIV
Hematocrit at least 38%	No Acutane or Proscar (1 mo.)	Human tissue treatment recipient
		IV drug abuse
Hemoglobin at least 12.5 g/dl	Pregnancy concluded at least 6 wks.	Jail >72 hrs.
	Tattoos (1 yr.)	
Clear skin exam (no needle tracks or Kaposi's sarcoma)	Free of gonorrhea and syphilis (1 yr.)	Major organ donor
	No Soriatane (3 yrs.)	Malignancy (blood or recurrent tumor)
	Over-the-counter drugs, herbs, prescriptions (refer to M.D.)	Travel to malaria endemic area/ Risky Sex

## Collecting a single unit

Place the donor in a reclining chair or bed. Wash and glove your hands. Apply a tourniquet briefly around the donor's bicep. Choose a vein in the antecubital fossa. Disinfect the area. Insert an 18g or 20g needle bevel up into the vein. Remove the tourniquet. Secure a blood bag containing 63 MI of CPDA-1 anticoagulant to the arm and collect 520 mL. Milk the bag very gently to mix the preservative with the blood. Collect three 10 mL red stoppered blood tubes and one lavender stoppered tube from the line for testing ABO, Rh, antibodies, CBC, Hepatitis B and C, HIV and syphilis. Remove the cannula. Apply direct pressure over the puncture site. Apply a bandage or cotton and hypoallergenic tape for two hours. Give the patient a sugary drink and cookies to replace volume and discourage syncope. Monitor the patient at least 10 minutes before discharging him/her.



## Donations

Autologous and directed donations:

- *Autologous donation:* The patient banks his/her own blood at least two weeks before an elective procedure for self-use to avoid infection, transfusion reaction, or cancellation of the surgery because a rare blood match cannot be found from an anonymous donor. Irregular antibodies and infectious agents, except Hepatitis B and HIV, are allowed. Discard the blood if it is not used. Do not give it to another patient.
- *Directed donation:* A donor known to the patient donates blood specifically for a procedure. Directed donation creates confidentiality problems because the known donor may not want to answer the screening questionnaire truthfully, so the quality of the sample is compromised. Directed donation may lead to alloimmunization for transplant recipients, hemolytic disease of the newborn when the father donates to the pregnant mother, and transfusion-related graft versus host disease (TAGVHD). The white cells must be irradiated to prevent fatal TAGVHD, making the shelf life only 24 hours, so this is a very expensive method.

## Collection terms

The following terms regarding blood collection:

- *Apheresis:* A donor gives one blood component (e.g., plasma, platelets). The rest of the blood is transfused back by hemapheresis.
- *Blood products:* Cost-effective technique to divide a single unit of whole blood into albumin, clotting factors, gamma globulins, irradiated product, leukocyte-depleted red blood cells, packed red cells, plasma, platelets, washed red cells, and white cells. Products have a longer shelf life and less chance of transmitting disease.
- *Cell saver:* Collect blood during surgery from the operating field, wash it, mix it with sterile normal saline, and return it to the patient. Do not use a cell saver in the presence of cancer or infection.
- *Hemodilution:* Collect blood prior to an elective surgical procedure. Replace it with plasma expander. Red blood cell loss during surgery should be minimal, and the patient will receive his/her own RBCs back afterwards.
- *Wound drainage:* Drain blood that has leaked into cavities and joints, filter it to remove clots and tissue shreds, and return it to the patient. Wound drainage cannot remove inflammatory chemicals (cytokines).

## Blood component products

Blood product	Use	Single Unit Volume	Shelf Life	Temperature Setting
Whole blood	Trauma resuscitation	520 mL	35 days	+4°C
Cryoprecipitate	Hemophilia hypofibrinogenemia von Willebrand disease	15 mL	1 year	+18°C
Fresh frozen plasma	Bleeder with coagulation deficiency trauma (with RBCs)	225 mL	1 year	+18°C
Platelet concentrate	Platelet dysfunction Thrombocytopenia	50 mL	5 days	+20°C
Platelet pheresis		300 mL	5 days	+20°C

## Red blood cells

Manually separating a single unit of blood into red blood cells and washed red blood cells, including shelf life, correct storage temperature, volume, and uses:

- *Red Blood Cells:* Centrifuge a 520 mL bag of whole blood mixed with CPD anticoagulant and separate the plasma from the red cells. Add 100 mL of Optisol or AS5 (adenine, dextrose, mannitol, and sodium) to the cells to extend the shelf life to 42 days between 1°C and 6°C. One unit contains approximately 180 mL of red cells, 30 mL of plasma, and 100 mL of preservative (310 mL total). Red cells increase the hematocrit 3% and the hemoglobin by 1 gm/dl in anemic patients when B<sub>12</sub>, iron, and folate are ineffective.
- *Washed Red Blood Cells:* Separate the red blood cells from whole blood, as above. Open the bag. Use sterile normal saline to wash away the plasma. About 20% of the red cells will be lost during washing. Hematocrit and hemoglobin will not rise as much as with unwashed cells. Use within 24 hours for patients who have had previous transfusion reactions (even though they received antihistamines beforehand), or paroxysmal nocturnal hemoglobinuria, or complement mediated immune hemolysis.

## Specimen quality

All blood bank specimens must be labeled at the bedside with the patient's full name or the Emergency Department identification number if unidentified – initials are unacceptable. The patient must wear an identification band at collection and transfusion times – it is insufficient to tape it to the bed. The band must have the patient's name and a unique identification number (Blood Bank identification number, hospital number, health insurance number, or unique lifetime identifier) in case there is a patient with a similar name. The requisition must bear: The names of the collector and the person who positively identified the patient; collection date and time (in case antibodies develop); ordering doctor's name; amount and type of blood requested; patient's date of birth (if

known); relevant patient history (e.g., pregnant and bleeding; signs of transfusion reaction). All blood must not be hemolyzed. Donor blood must not be clotted, lipemic, or have foreign objects in it. Reject the sample if all these conditions are not met.

### **Blood Bank agglutination tests**

The most common agglutination test is blood typing (A, B, O), also called hemagglutination, to prevent blood transfusion reactions. Antibodies crosslink red blood cells that are coated with antigen. If the blood is not agglutinated, it will look like a solid red dot at the bottom of an incubation well. If the blood is agglutinated, it will look diffuse or O-shaped at the bottom of an incubation well. Titer the antibodies by making serial dilutions of serum in incubation wells, and measure the highest dilution still capable of agglutinating the blood. Most agglutination reactions take place within 2 minutes. The incubation rows are marked Neg and Pos for the controls, and 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024 for the serial dilutions. Look along the row for the last O-shape before the blood becomes solid red dots. If the last O is at 64, then report the titer as 1:64.

### **Gel agglutination tests**

Gel agglutination tests are replacing tube tests because they do not require cell washing. They are easier to read and more reproducible than tube methods of antibody identification. Gels detect weak antibodies better than tube methods, and can use either serum or plasma. Reactions remain intact 3 days if parafilm and refrigerated upright. The test is performed on a plastic card with 6 microtubes containing dextran acrylamide gel with your choice of broad-spectrum anti-IgG, anti-A, anti-B, anti-AB, anti-D, anti-E, anti-C, anti-c, or anti-e. Pipette 50 microliters of 0.8% red cell/saline suspension and 25 mL patient blood onto the gel. Incubate 15 minutes at 37°C. Centrifuge for 10 minutes at 70 g. Read the card on a white background. If blood sinks to the bottom of the tube, the result is negative. If the blood agglutinates near the gel surface, the result is a strong positive reaction (4+). If the cells disperse along the length of the tube, it is a moderate reaction.

### **SPRCA**

Solid phase red cell adherence assay (SPRCA) detects antibodies to cytomegalovirus, platelets, red cells, and syphilis. Place known phenotype red cells in wells on a V-bottomed microtiter plate. Add the patient's plasma or serum. Incubate at 37°C. The time will vary according to the type of antibody you are trying to find, so follow the manufacturer's directions exactly. Wash away unbound proteins by irrigating the wells with phosphate buffered saline (PBS). Add IgG-coated indicator red cells to each test well. Centrifuge to crosslink. If the indicator red cells stick to the sides of the well, report the test result as positive. If the red cells do not stick to the well, report the test result as negative.

## **ABO typing discrepancies**

An ABO discrepancy occurs when the antigen/antibody reaction you expect is unclear (weak reaction). It is most common in newborns and the aged. Check for clerical errors, like transposing entries on the results worksheet. Check that the sample and reagent bottles are correctly identified and uncontaminated. Repeat the test with a lab buddy, and make sure you add the reagent before the cells. Do not shake the tube vigorously after centrifuging, thereby breaking up the agglutination. IgM antibodies work best in cold temperatures, so if the ABO test is too warm, it may produce a false-negative. Do not overwhelm the reagent antibodies with too much of the patient's blood. Check for dirty test tubes, hemolysis and Rouleaux formation. If you cannot clarify the discrepancy with these checks, the only blood that you can release for transfusion to a recipient is O– (universal donor). If the discrepancy is found in a donor's blood, do not label it with a blood type until you get a clear test result.

## **Lupus inhibitor**

Lupus inhibitor is an important antibody test to check when PT and PTT tests are too long. Phospholipid is used in PT and PTT tests to check how fast a patient clots. Lupus inhibitor antibody (LA) acts against the phospholipid and will give a falsely extended clotting time result. Lupus inhibitor might give a false positive VDRL (syphilis) result, or indicate the patient will have repeated miscarriages in the second and third trimester of pregnancy (habitual aborter) if anticardiolipin autoantibody is also present with it. If lupus inhibitor alone is present, it alerts the doctor that this patient might have a thromboembolism, rheumatoid arthritis, Systemic Lupus Erythematosus (SLE), or Raynaud's syndrome, and further tests are warranted.

Mix one milliliter of the patient's plasma from a blue stopper tube with one milliliter of normal plasma, and repeat the PTT test. If an inhibitor is present, the PTT will be longer than the normal range of 60 to 70 seconds.

## **Platelet Antibody Detection Test**

If a patient has purpura (purple spots on the skin from bleeding), the cause must be located. It could be thrombocytopenia -- too few platelets (thrombocytes) circulating in the blood, which diminishes clotting power and predisposes the patient to bruising, nose bleeds (epistaxis), bleeding gums, and gastric ulcers. This can be because the platelets are being destroyed prematurely (sooner than 10 days) in the bloodstream (intravascular), or by the liver and spleen (extravascular), or they are not being produced fast enough by the bone marrow because of anemia, cancer, drugs, or infection. Less than 150,000 platelets per microliter of blood are too few. Patients with hemoglobinuria (hemoglobin in the urine) and purpura should have a Platelet Antibody Detection Test. The PLAI, ALTP, and PAIgG should all be negative.

## Compatibility tests

COMPATIBILITY TESTS		
Antigen	Antibody	Type
A only	Anti-B	Type A
B only	Anti-A	Type B
A and B	Anti-A and Anti-B	Type AB
No A or B	No Anti-A or Anti-B	Type O
Antigen	Antibody	Group
DD	Not usually performed	Rh+
Dd		Rh+
dd		Rh-

*Alloantibodies* can form on the red blood cells after a blood transfusion or pregnancy. Alloantibodies are detected with a screen that takes a whole day to complete. *Type and cross* ensures the donor's red cells are compatible with the recipient's serum. It takes 45 minutes. Take the crossmatched unit out of inventory and put it in the reserved refrigerator for up to 72 hours. Unnecessary blood crossmatching causes inventory shortages and elective surgery will be cancelled if the inventory runs short. If there is no time to crossmatch a trauma patient, O – blood is given without compatibility testing to prevent death. O – is the *universal donor*.

## DAT and automated cell washer

*Direct Antiglobulin Test (DAT)* is performed to diagnose blood transfusion reactions, hemolytic disease of the newborn (HDN), and hemolysis from drug or autoimmune reactions. *The ICE Centra-W cell washer by Thermo Scientific, the IBM 2991, and the Coulter CellPrep* are typical cell washers used before antiglobulin testing; they all work similarly. You can wash cells with saline and pellet them in about 80 seconds for twelve patients at the same time using a cell washer. The results are more easily repeatable than manual methods and produce less hemolysis. Use a cell washer before performing ABO, Rh, Coombs, cross-match, and DAT. Load the test tubes into the cell washer. Select the parameters on the touchpad. The machine will alert you if your solution levels are low, and when washing and centrifuging are finished. Read the results on the LED display. The centrifuge cups are autoclavable and the rest of the machine can be cleaned with 95% ethanol that can be vacuumed out.

## Transfusion reactions

Patients with transfusion reaction have burning at the IV site, rigors (severe chills and fever), back and joint pain, difficulty breathing, stomach upset, oozing wounds, apprehension, and then go into shock from circulatory collapse (hypotension). The chance of your patient having an allergic reaction to plasma proteins (itchy hives or anaphylaxis) is 1 in 500. The chance of your patient having a febrile reaction to white blood cells (chills and >1°C temperature increase) is about 1 in 10,000. The chance of your patient having a

transfusion-related acute lung injury (TRALI) from HLA in fresh frozen plasma is about 1 in 100,000. The chance of your patient having a hemolytic reaction from an incompatible blood group, with disseminated intravascular coagulation (DIC), renal failure, and death, is about 1 in 100,000. If the blood is stored improperly, your patient may get a bacterial infection (sepsis). Giving too much IV fluid and blood too fast can result in circulatory overload and damage to the left ventricle.

If suspected transfusion reaction is reported to you: Write the signs and symptoms on the patient's chart. Tell the nurse to stop the transfusion immediately and alert the attending physician. Request a urine sample to look for hemoglobinuria. Verify the patient's identity. Draw the patient's blood for STAT O<sub>2</sub> saturation, CBC with platelets, bilirubin, calcium, electrolytes, liver enzymes, pH, PT and aPTT. Take the donor blood bag back to the lab with you to verify that it is the correct unit. Repeat antibody screen, crossmatch, direct and indirect Coombs' tests on the donated blood. When you centrifuge the patient's blood for chemistry, note if the serum is pink, indicating hemolysis. If it is a transfusion reaction: Bilirubin peaks in 3—6 hours; hemoglobin drops in 1—2 days; hematocrit will not rise from pre-transfusion levels; eosinophilia, hypocalcemia, leukopenia, and pancytopenia may present. The doctor will order ECG, chest x-ray, acetaminophen, diphenhydramine, oxygen, corticosteroids, and perhaps furosemide, dopamine, epinephrine, and calcium gluconate, depending on the type of reaction.

### **RhoGAM testing of pregnant women**

If the mother is Rh- and the father is Rh+, as determined by Rh(D) tests, the infant is at risk for HDN. Antibody screen the mother's blood to find out if she acquired anti-D antibodies from a previous blood transfusion or a pregnancy. Arrange for the Rh- mother only to get a 300 mcg RhoGAM shot (RhIg, Rh immunoglobulin) intramuscularly at 26—28 weeks of pregnancy and again within 72 hours after her delivery. Do not inject the infant or father. RhoGAM prevents any Rh+ cells from the infant that have crossed over to the mother from causing her to make anti-D antibodies that will attack the infant and sensitize her for future pregnancies. Explain to the mother that she needs RhIg even if the pregnancy ends in a miscarriage, or is terminated because it is ectopic or aborted. If the pregnancy ends at 12 weeks or less, inject the mother with 50 mcg of MICRhoGAM. If the pregnancy ends at 13 weeks or more, use RhoGAM.

### **HDN**

HDN (hemolytic disease of the newborn) is also called erythroblastosis fetalis, alloimmunization, Rh immune hydrops, and fetomaternal hemorrhage. Affected babies have jaundice, hemolysis and hyperbilirubinemia (kernicterus). It may occur from an Rh-mother's anti-D antibodies attacking an Rh+ fetus, or from Duffy (Fya), Kell (K and K), Kidd (Jka and Jkb), and MNSs antibodies. Sensitization occurs during abortion, abruptio placenta, amniocentesis, cesarean section, chorionic villus sampling, cordocentesis, delivery, ectopic pregnancy, and toxemia. HDN affects 1% of pregnancies, and kills 50% of untreated babies. Use indirect Coombs, DAT, Rh antigens, and Kleihauer-Betke acid elution

technique to determine the amount of fetal red blood cells circulating in the mother's blood. Find fetal blood group by PCR (cordocentesis), if the father is heterozygous. Use a spectrophotometer for bilirubin and plot a Liley curve for the weeks of gestation; if Zone 3 is reached, or Hct <30%, transfuse the fetus in utero with irradiated, leukoreduced, O-packed red cells with no Kell antibodies or cytomegalovirus, crossmatched with the mother's serum. Give 10 mL per week of gestation, minus 20.

### **Calculating antibody titer**

A titer measures how many times a blood sample must be diluted with saline before an antibody can no longer be found in it. Warm blood, saline and a pipette to 37°C for 10 minutes to prevent cold agglutinins from forming Rouleaux. Spin the blood in a centrifuge placed in a 37°C incubator. Pour off the serum. Test the undiluted serum for antibody/antigen reaction against a control. Then make serial dilutions. For example, dilute 1 mL of serum with 1 mL of saline for a total volume of 2 mL. The dilution is ½, or 1:2. Pipette off 1 mL of this dilution into an aliquot tube. Add 1 mL of saline, and it becomes a 1:4 dilution. If you dilute up to 1:32 and get no reaction, the end-point titer is 16.

Patients co-infected with HIV and syphilis have a prozone phenomenon, where the undiluted serum does not agglutinate (false-negative) or has very little agglutination at low dilutions, but agglutinates more at higher dilutions because of excess antibodies.

### **Cryopreservation**

After 42 days, refrigerated blood expires. Cryopreservation keeps rare blood types up to 10 years. Cryopreservation is commonly used to keep blood on hand for patients who have antibodies to common antigens, IgA deficiency, multiple alloantibodies, and Rh- blood. Wash the red blood cells with isotonic saline in a cell washer. Deplete them of plasma and leukocytes. Incubate the red blood cells in 40% glycerol (antifreeze). Place them in a freezer set at -60°C or lower. When the time comes to use the red cells, thaw them, wash off the glycerol, and suspend them in saline. Use within 4 hours to prevent the patient from contracting bacterial sepsis.

### **Using blood warmers**

Ordinarily, do not warm just one or two units of blood or blood products before transfusion if you are giving them over several hours. However, if the patient receives several units in a short time, unwarmed bags cause hypothermia. Blood warmers are used in the O.R., Emergency and Trauma units for a massive transfusion. It also warms saline and other IV fluids given together with the blood. Some common brands of blood warmers are Fenwal, Gorman-Rupp, HOTLINE, Medi-Temp, and Thermal Angel. Uncap the luer lock from the heater's input. Attach the IV tubing. Uncap the luer lock from the heater's outlet. Connect the IV extension. Connect the IV line to the blood or fluids. Prime the line to prevent air bubbles. Connect the warmer to a power outlet or portable battery. Check the

“ON” light to confirm the heater works. Adjust the flow rate with the roller clamp on the IV. When the blood/fluid warms to about 100.4°F, couple the IV catheter into the patient’s line.

### **Lui Freeze-Thaw Elution Technique**

Use 2 mL EDTA blood or 1 mL clot from a red tube. Add 37°C physiologic saline. Mix by inverting. Centrifuge 1 minute at high speed. Pipette off and discard supernatant. Wash the cells three more times. Add 2 drops of supernatant to a clean tube. Mix with one drop of anti-human globulin and one drop Coombs’ control cells. Centrifuge and look for agglutination with a lighted view lamp. If agglutination is weak, wash the cells twice more and repeat the test. If agglutination is strong, no antibodies remain. Place 15 drops of washed cells and 3 drops of saline in a clean tube. Invert and rotate. Dry-ice for 20 minutes. Thaw in hot water. Centrifuge 5 minutes at high speed. Save eulate in a clean tube. Prepare three clean tubes with washed A, B, and O blood. Add 3 drops of eulate to each tube. Incubate 30 minutes at 37°C. Wash all tubes 3 times. Add a drop of anti-human globulin to each. Centrifuge, resuspend, and look for agglutination.

### **Prioritizing workload and needs**

How to triage and anticipate your workload for Blood Bank patients and how the surgical team helps conserve blood to meet the needs of your hospital:

- *First priority:* Emergent trigger patients (trauma with acute isovolemic anemia or hemoglobin 5 g/dl). E.R. notifies you of incoming accident victims or disaster casualties because emergency transfusions deplete the O- supply.
- *Second priority:* Surgical patients who lose more than 1200 mL of blood during surgery, or who have staged surgery. Cardiac patients take 80% of your blood inventory. Anticipate transfusing the underweight, anemic, chronically diseased, or cancerous. Elective surgery is cancelled if emergent patients use the blood inventory.
- *Third priority:* Regular users of coagulation factors. Hemophilia Clinic advises you of the day’s requirements, so you can contact suppliers for stock, or split whole blood into products.

Call your supervisor and the relevant nurse manager if you anticipate a shortage. The surgical team conserves blood by: Giving patients erythropoietin or hemodilution before surgery; using cell savers, hypotension, electrocautery, and lasers during surgery; and administering antifibrinolytics after surgery. Suggest autologous donations for patients having surgery in the coming month. Suggest delaying surgery for anemic patients, pending treatment with iron, B<sub>12</sub>, or folate.

### **Receiving unused or returned blood**

Keep a permanent record of the final fate of any blood you issue (transfusion, disposal, reaction/retesting, returned to inventory). When the ward transfuses a unit, a traceability slip from the label is sent back to Blood Bank to confirm it was used. Scan its bar code into



the computer to complete the chart. If a unit needs to be returned, record the reason, e.g., patient expired. Make sure the tamper-proof, traceable label is still attached firmly to the unit of blood. Check the expiry date. Confirm the blood bag is not leaking, and that no clots, hemolysis, or foreign objects are present. The nurse returning the unit signs off the return slip and you must co-sign. Place a copy in the patient's chart. Once in the lab, check the time of issue and temperature of the bag to determine if it should be discarded or returned to inventory. Account for all blood on a daily basis. If you cannot, make a non-return report to your supervisor.

### **Leukocyte-depleted cells**

Blood processing labs filter out leukocytes from red blood cells to transfuse patients when:

- Cytomegalovirus (CMV) must not be transmitted to pregnant women or their fetuses
- Chlamydia pneumoniae must not be transmitted to frail or immunosuppressed patients (cancer chemotherapy, hemodialysis, HIV)
- Human leukocyte alloimmunization reaction (HLA) must be avoided in organ transplant patients
- At least two non-hemolytic febrile transfusion reactions have occurred in this patient, despite antihistamines being administered beforehand
- The patient has two or more refractory platelet transfusions (unsatisfactory platelet increase of less than 5,000 mCL) because of acute promyelocytic leukemia, DIC, hepatic veno-occlusive disease, sepsis, or TTP-HUS

Unfortunately, leukocyte filtration reduces the number of RBCs by 15%, so the transfusion will not be as effective for treatment as plain or washed red blood cells. It is expensive to filter the blood with a Sepacell R-500 leukocyte reduction filter by Asahi. Some donated blood does not filter well, cannot be transfused, and cannot be returned to the donor pool.

### **Irradiated blood**

Irradiated blood products prevent fatal transfusion-associated graft-versus-host disease (TAGVHD). These patients get irradiated blood transfusions: Bone marrow recipients (and donors who require autologous transfusion); direct donors; family members; fetuses and premature babies; glioblastoma; HLA-matched platelet recipients; Hodgkin's disease; leukemia; lymphoma; neuroblastoma; T-cell deficient. Patients with intact immune systems can destroy foreign lymphocytes and do not need irradiated blood. Generally, you do not need to give irradiated blood to patients with AIDS, aplastic anemia, most types of cancer, humoral immunodeficiency, prednisone use, or solid organ transplants. Fresh plasma only should be irradiated, not cryoprecipitate or fresh frozen plasma. The latter two products have no known association with TAGVHD. Use 2500 rads of gamma radiation for 12 minutes in a dual x-ray field to make the leukocytes unable to reproduce by cell division, and to make them non-reactive to the recipient.

## Antibody terms

The following terms regarding antibodies:

- *Clinically significant antibodies:* Warm antibodies that react at temperatures over 35°C. They cause hemolytic disease of the newborn or transfusion reactions. Clinically significant antibodies include: Duffy antibodies; Kidd antibodies; Kell antibodies; Rh antibodies reactive by IAT; anti-S and anti-s antibodies.
- *Insignificant antibodies:* IgM antibodies that usually appear the first time the blood/saline suspension is centrifuged. Insignificant antibodies include: Anti-A1, Anti-Le, Anti-M, Anti-N, Anti-P1, and high titer low-avidity antibodies (HTLA).
- *Monospecific reagent:* Reacts with only one type of antigen, like anti-IgG.
- *Polyspecific reagent:* Antibody blend that reacts with more than one antigen, like polyspecific anti-human globulin reagent to detect both IgG and C3 complement. If you get an equivocal result with a polyspecific reagent, retest with monospecific reagents.
- *Pretransfusion tests:* ABO with anti-A and anti-B; Rh(D) with anti-D reagent; antibodies by hemagglutination or hemolysis; antigens C, c, D, E, e, Fya, Fyb, Jka, Jkb, K, k, M, N, S, and s. These can all be done by computer crossmatch.

# Microbiology

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## Bacteriology

### Micro-organisms

Some micro-organisms that are harmless, commensal, or beneficial:

- *Aquificae* bacteria dislike oxygen, prefer water at least 85°C, eat inorganics, and live in hot springs, ocean vents, and sulphur pools
- *Chlorobia* bacteria are dark green anaerobes that need light, so they live just under the surface of shallow lakes, and produce sulphur
- *Chrysiogenetes* bacteria eats arsenic and lives in low oxygen areas
- *Fibrobacter* are in the stomachs of ruminants (camels, cows, goats, and sheep) to digest fibrous plants.
- *Lactobacilli* live in buttermilk, cheese, and yogurt, and keep the number of pathogens in human guts low by competing with them (exclusion)
- *Leuconostoc mesenteroides* bacteria preserve food by pickling it (e.g., cassava, olives, sauerkraut, sausages, sour dough bread) and killing *Listeria*
- *Sachharomyces cerevisiae* yeast ferments grapes into wine
- *Sphingobacteria* are gliding, gram-negative rods that live in soil and water and break down rotting vegetation
- *Thermomicrobia* are green bacteria that prefer warm temperatures found in compost, hot springs, ocean vents, and peat bogs

### Phyla

These phyla are predominantly pathogens. Examples of a disease each cause:

- *Bacteroids Xenobacteria1*: Usually harmless or commensal flora; if host's condition weakens, they become parasites
- *Cyanobacteria*: Fever and intestinal cramps from water contaminated with blue-green algae
- *Firmicutes*: *Clostridium* (botulism, colitis, gas gangrene, tetanus); *Listeria* (food poisoning); *Mycoplasmas* (pneumonia, pelvic inflammatory disease); *Staphylococcus* (wound sepsis); *Streptococcus* (endocarditis, flesh-eating disease, and meningitis)
- *Flavobacteria*: Bacterial cold-water disease and rainbow trout fry syndrome
- *Fusobacteria*: Pyorrhea: Lemierre's syndrome (septic blood clot in the jugular vein that breaks up and causes abscesses in distant parts of the body, like the brain, joints, kidney, and liver)
- *Planctomycetes*: *Chlamydia trachomatis* (blindness, genital infections, urethritis); *Chlamydophila pneumoniae* (pneumonia)

- *Proteobacteria*: E-coli (food poisoning); Helicobacter (stomach ulcers); Neisseria (gonorrhoea); Salmonella (typhoid); Vibrio (cholera); Yersinia pestis (plague)
- *Spirochaetes*: Borrelia burgdorferi (Lyme disease); Borrelia recurrentis (relapsing fever); Leptospira (Leptospirosis); Treponema (syphilis and yaws)
- *Verrucomicrobia*: Verruca vulgaris (warts on feet and hands); Human Papilloma Virus (genital warts)

## Bacteria

Examples of these bacteria types:

- *Aerobic*: Thrive in the presence of oxygen, like neisseria, pseudomonas, and staph; obligate aerobes congregate at the top of a test tube
- *Anaerobic*: Live without oxygen, like Bacteroides fragilis, Peptostreptococcus, and Clostridia; obligate anaerobes congregate at the bottom of a test tube
- *Facultative*: Can live with or without oxygen, like yeast, Bacillus cereus, Salmonella, Shigella, and Staphylococcus aureus; congregate all along a test tube
- *Curved gram-negative*: Campylobacters and Vibrios
- *Fastidious Gram-negative*: Can only grow in special conditions, like Acinetobacter baumannii, Bordetella pertussis, Brucella, Campylobacter, H. pylori, Legionella pneumophila, and Pasteurella multocida
- *Gram-positive*: Hold crystal violet stain because of their thick peptidoglycan walls, like anthrax, diphtheria, and strep; lack the outer membrane that protects gram-negatives, so are usually more susceptible to antibiotics than gram-negative bacilli are.
- *Nonfermentative*: Do not eat glucose, like Laribacter hongkongensis; smell fruity; resist antiseptics and antibiotics; inhabit catheters, dialysis fluid, and nebulizers.
- *Oxidase-positive fermenters*: On MAC agar, fermenters are red and nonfermenters are colorless.

Bacteria require these things to eat, grow, metabolize, and reproduce:

Acid/base balance (pH) that varies according to species; amino acids to make proteins, Calcium cation, and to make endospores; carbon from organic compounds or carbon dioxide (CO<sub>2</sub>) to form most cellular material; energy source according to species, like light for phototrophs; inorganic phosphates (PO<sub>4</sub>) for nucleic acids, nucleotides, phospholipids, and teichoic acids; iron for cytochromes; magnesium cation; nitrogen for amino acids, nucleic acids, and coenzymes; organic sulfur for coenzymes; oxygen (O<sub>2</sub>) if aerobic, or carbon dioxide (CO<sub>2</sub>) if anaerobic; potassium cation; pyruvic acid and purines for DNA and RNA; temperature that is right for the species, like 50 — 80°C for thermophiles and 10 — 15°C for psychrophiles; trace elements, like copper and zinc, that vary according to the species; vitamins for enzymes; water.

## Normal flora

Normal flora is a combination of helpful micro-organisms that live in the gut and on the skin. They include about 200 types of bacteria, and a few fungi, protists, and archaea. They have a symbiotic or commensal relationship with their host, whereas pathogens have a

self-seeking relationship. Flora helps us by: Competing with pathogens for resources; digesting food; making vitamins (e.g., K and B<sub>12</sub>). Flora only becomes dangerous if: The immune system is compromised because it is depressed (e.g., AIDS or stress); trauma allows flora that is normal on the skin to enter the flesh, where it can become a pathogen; overgrowth occurs (e.g., candida normally present in the vagina overgrows to become a yeast infection after broad-spectrum antibiotics are taken for bronchitis). Some examples of flora are: Actinomycetes; Corynebacteria; Lactobacilli; Proteus; Veillonellae.

#### How to collect a normal flora swab and plate it:

1. Wash and glove your hands.
2. Explain to the patient what you are going to do.
3. Rotate a sterile cotton swab on its sides over a 4 cm diameter area of the patient's skin.
4. Open a sterile agar plate. Do not touch the media with your hands or breathe on it.
5. Touch the cotton swab to one place in the agar.
6. Close the plate cover immediately.
7. Discard the used swab in a biohazard container.
8. Use an indelible marker to label the plate cover with the patient's name, date, skin swab, body area, and doctor's name.
9. Once in the lab, streak the inoculate three times across the plate with a sterile loop. Avoid tearing the agar with the sharp edge of the loop.
10. Place the plate in an incubator set at 37°C for at least 24 hours, and up to 48 hours.

#### **Isolating pure bacteria culture**

How to isolate a pure bacteria culture on an agar plate:

1. Wash and glove your hands.
2. Look for three isolated colonies of bacteria (separated dots) on the primary streaked agar plate, which have grown for 24 to 48 hours in an incubator.
3. Sterilize an inoculating loop by passing it through a Bunsen burner flame and let it cool for 5 seconds.
4. Obtain three sterile agar slants; do not breathe on them or touch the media with your gloves.
5. Open the lid on the first new slant.
6. Pick up an isolated colony from the primary agar with the loop and plant it with three streaks across the first new slant.
7. Close the lid on the first new slant.
8. Sterilize the loop again.
9. Inoculate the second and third slants as you did the first slant, making sure to sterilize the loop between inoculations.
10. Use an indelible marker to label the new plate lids with the patient's name, date, source, and doctor's name.
11. Incubate the three new plates at 37°C for 24 hours.

## Smears

How to identify bacteria using a microscopic smear with Gram stain:

1. Label a glass slide with pencil on the frosted end.
2. Place a drop of water on the slide.
3. Pick up a bacteria colony from an agar plate with an inoculating loop.
4. Mix the colony in the water drop until it becomes a milky suspension.
5. Air dry the smear.
6. Heat fix the smear by quickly passing it over a Bunsen burner several times.
7. Do not lyse the cells by cooking them – if the slide feels too hot when placed on the back of your hand, then heat killed the bacteria.
8. Submerge the slide in crystal violet stain for one minute.
9. Remove the slide from the crystal violet.
10. Rinse slide with water.
11. Submerge the slide in Gram's iodine solution for one minute.
12. Rinse slide with water.
13. Blot slide with tissue.
14. Hold the slide at an angle. Decolorize with 95% acetone-alcohol-safranin solution until it runs clear.
15. Rinse slide with water.
16. Air dry or blot before reading.

How to make a smear with acid-fast stain to detect tuberculosis:

1. Label glass slide with pencil.
2. Place one drop of water on slide.
3. Pick up a bacteria colony from an LJ egg plate with an inoculating loop.
4. Mix colony in water drop until it becomes a milky suspension.
5. Air dry smear.
6. Heat fix smear by quickly passing it over a Bunsen burner several times.
7. Do not lyse the cells by cooking them – if the slide feels too hot when placed on the back of your hand, then heat killed the TB.
8. Submerge slide in Kinyoun carbol fuchsin stain for five minutes.
9. Remove slide from Kinyoun stain.
10. Hold slide at an angle. Rinse slide with deionized water decanted in a squirt bottle.
11. Decolorize with a 70% ethanol/0.5% hydrochloric acid solution until it runs clear.
12. Rinse slide with deionized water.
13. Submerge slide in 1% methylene blue stain for 1 minute.
14. Rinse with deionized water.
15. Air dry or tissue blot.
16. Look for red bacteria with oil immersion lens.

### How to make a smear with methylene blue stain to look at normal flora:

1. Label a glass slide with pencil on the frosted end.
2. Place one drop of water on the slide.
3. Pick up a bacteria colony from an agar plate with an inoculating loop.
4. Mix the colony in the water drop until it becomes a milky suspension.
5. Air dry the smear.
6. Heat fix the smear by quickly passing it over a Bunsen burner several times.
7. Do not lyse the cells by cooking them – if the slide feels too hot when placed on the back of your hand, then heat killed the flora.
8. Submerge the slide in methylene blue stain for one minute.
9. Remove the slide from the methylene blue stain.
10. Hold the slide at an angle.
11. Rinse the slide with water.
12. Air dry or tissue blot the slide.
13. Examine the smear with an oil immersion lens.

### How microbiology smears can give false results and how you can avoid this:

- *Too thick:* If you make a microscopic smear to identify the shape of bacteria and it is too thick, then you may get a false-positive reaction because the bacteria are concentrated. Deep-lying bacteria on a thick smear are protected from decolorization with acetone, so a gram-negative appears to be a gram-positive.
- *Over-washing:* Over-washing a smear with excessive acetone and water can decolorize bacteria too much, so gram-positive appears gram-negative or gram-variable. Be skeptical about the decolorization technique used if you find gram-negative cocci in a smear from a normal skin swab. If in doubt, place two smears on the same slide for side-by-side comparison – one smear containing known bacteria, and one with the bacteria you are trying to identify.
- *Too thin:* If you make a microscopic smear too thin, then you will spend too long looking at the slide for widely spread bacteria and may report a false-negative.

### **Microbiology stains**

Some common types of microbiology stains and the types of diseases, cells or structures each is used to identify:

- *Acid-fast* stain turns tuberculosis bacilli red.
- *Periodic acid-Schiff (PAS)* stains carbohydrates, collagen, fibrin, and mucin purple.
- *Ryu* flagella stain turns the fragile flagella (arms) that propel motile and anaerobic bacteria blue-violet.
- *Silver* stains turn proteins black.
- *Toluidine* blue stain turns the background blue and the cells reddish purple; used for *P. carinii* cysts and mast cells.
- *Warthin-Starry* stain turns the background yellow and spirochetes and *H. pylori* black.
- *Weber* stain turns the background light green and microsporidia spores dark violet.

- *Wright-Giemsa* stain turns red blood cells reddish yellow, neutrophils pink with lilac granules, basophils purple, lymphocytes blue, and platelets violet.
- *Ziehl-Neelson* stain turns the background blue and acid fast bacilli red.

### **Identifying anaerobic bacilli**

To collect anaerobic bacilli that live without oxygen, use transport media made from 0.1% blood agar or cooked meat and thioglycolate reducer. Syringe pus from the wound or swab the infected tissue. Place the swab into the media immediately and cap it tightly to avoid contact with oxygen. Use a Gas-Pak jar, or quickly move the transport media into an airtight jar with nitrogen and 10% carbon dioxide atmosphere and CDC differential agar, and create a reduced environment with a hydrogen electrode. After a few days, use a gas chromatograph to analyze byproducts from the spent media. You can also use hydrogen sulfide, maltose fermentation, or milk proteolysis tests for anaerobic cocci.

### **Sensitivity testing**

Sensitivity testing is also called culture and susceptibility testing (C&S). Place body fluid or tissue on media and incubate at body temperature (37°C) for 24 — 48 hours to see if any micro-organisms grow on it. If there is growth, distinguish normal flora from pathogens by chemical and enzyme tests. Inoculate pathogens with antimicrobials (antibiotics, antifungals, or antivirals) to see if they can be killed (susceptible) or cannot be killed (resistant). If the antimicrobial that works best requires high doses (intermediate), it is likely to be toxic to the patient. The doctor initially prescribes the antimicrobial to which the pathogen is susceptible, except if the patient is allergic to it. Then the doctor consults with a pharmacist and microbiologist to choose the least toxic alternative, but the intermediate dose may need to be given over a long time and the patient may suffer side-effects. If the pathogen is resistant to many antimicrobials, then expensive intravenous combination therapy may be the only effective treatment.

### **Kirby-Bauer manual method**

How to test a specimen for sensitivity using the Kirby-Bauer manual method:

1. Turn on a Bunsen burner flame for five minutes to clear your work area.
2. Wash and glove your hands.
3. Sterilize an inoculating loop in the flame; let cool five seconds.
4. Take a pure culture from the incubator and remove its lid.
5. Open a sterile agar slant; do not touch the media with your hands or breathe on it.
6. Inoculate the new slant with the pure culture by streaking across the whole plate once, then rotate it and streak again so that the bacteria are evenly distributed across the agar.
7. Sterilize the loop again; let cool five seconds.
8. Gently press numbered antibiotic discs into the new slant with the loop without tearing the agar.



9. Cover the new slant with its lid.
10. Use an indelible marker to label the new slant with the patient's name, date, source, and doctor's name.
11. Incubate the new slant at 37°C.
12. Inspect the slant at 24 hours for growth.
13. Read at 48 hours.

### Using a CAMP reaction

Small labs may not be able to afford a Bacitracin sensitivity test or the newer PYR test to identify hemolytic strep. Try the CAMP method instead: Use a cotton swab to inoculate a plate of sheep's blood agar with one streak of the suspected Strep colony. Use a clean cotton swab to make a single streak of *Staphylococcus aureus* across from it. Make sure the two bacteria types do not touch. Use *Strep agalactiae* as the positive control, and Group A or D strep as the negative control. Keep at room temperature in a normal atmosphere. Group B strep produce CAMP factor, which increases the hemolysis of red blood cells.

### Identifying strep

Cooked blood agar (CA) when mixed with blood agar (BAP) can support strep, which are more difficult to grow than staph because they require more nutrients and less oxygen. Streak the chocolate agar plate, then stab the loop into the media to increase the streptolysin O activity. Use a CO<sub>2</sub> incubator or a candle jar to produce a 5% CO<sub>2</sub> atmosphere similar to the human body. No halo around the colony is  $\mu$ -hemolysis (no hemolysis). A greenish halo around the colony is  $\nabla$ -hemolysis (partial). A clear area around the colony is  $\exists$ -hemolysis (full). The Bacitracin sensitivity test identifies  $\exists$ -hemolytic strep of Lancefield group A by a faster method than counter-electrophoresis. Place three paper Taxo A discs impregnated with Bacitracin in concentrations of 0.025, 0.7, and 2.5 IU on a culture media with a strep colony. Measure the zone of inhibition. Any clear zone is considered sensitivity (S2). Growth right up to the antibiotic disc is resistance (R2). 14mm is the critical level (S1 or R1).

### Catalase and coagulase tests

The difference between catalase and coagulase tests used to distinguish between staph and strep infections:

- *Catalase*: Staph produce catalase; strep do not. Confirm a pure *Staphylococcus aureus* culture by latex agglutination and Gram-positive stain. When it is 18–24 hours old, pick up staph from the middle of the colony on a loop. Do not pick up any agar, which invalidates the test. Place staph on a glass slide and cover with 3% hydrogen peroxide. If it bubbles off, it is staph.
- *Coagulase*: Staph produces the enzyme coagulase; strep do not. Confirm a pure *Staphylococcus aureus* culture by latex agglutination and Gram-positive stain. Pick up staph from the middle of the colony on a loop. Mix staph into a citrated (blue stoppered) tube of blood. As a control, take another organism known not to be

staph, and place it in another blue tube. Cover the tubes. Incubate four hours at 37°C. Tip the tubes and look for clot formation. Coagulase produced by staph reacts with coagulase-reacting factor in plasma and clots it. If there is still no clot after 18 hours, the test is negative.

### **Optochin versus bile solubility tests**

The differences between the Optochin sensitivity test and bile solubility test, which are used to distinguish pathogenic *Streptococcus pneumoniae* from other  $\alpha$ -hemolytic viridans strep that may be normal flora:

- *Optochin Test:* Take a sheep's blood agar plate that shows a greenish halo around the colony, indicating  $\alpha$ -hemolysis, and inoculate an isolation plate from it with a sterile loop. Place an Optochin-impregnated disc on the isolation plate before incubating it at 37°C. If you see an inhibition halo around the colony that measures 14mm or larger, then report the result as pathogenic *Streptococcus pneumoniae*. If the bacteria grow right up to the disc, or less than 14 mm, then the colony belongs to the viridans variety, like *Streptococcus mitis*.
- *Bile Solubility Test:* Add desoxycholate bile salt to a strep colony that is growing on agar or in broth. (The broth will be cloudy.) Bile salt will lyse *Streptococcus pneumoniae*, and the broth will clear, or an area on the agar touched by the salt will clear. If the colony stays visible, or becomes cloudy, then it is viridans strep.

*Streptococcus pneumoniae* causes bacterial pneumonia, bacteremia in babies, and middle ear infections.

### **Oxidase test**

Oxidase tests are usually performed to detect *Campylobacter*, *Moraxella*, *Neisseria*, *Pasteurella*, and *Pseudomonas*, which all produce cytochrome oxidase enzymes or indophenol oxidase. Use a plastic loop to smear a colony on one of the windows in a DrySlide, which has filter paper impregnated with substrate. Do not use a metal loop, because the oxidation from sterilizing it in a Bunsen burner causes a false-positive. If the colony is positive, then it will darken (oxidize) the window immediately with blue indophenol. Read the result at once, because even a delay of five minutes could cause the oxidation reaction to fade, giving a false-negative result. Use *Pseudomonas aeruginosa*, *Aeromonas* as the positive control.

### **Minimum Inhibitory Concentration test**

MIC is a visual test that measures the lowest antimicrobial concentration it takes to inhibit the growth of pathogens. Antimicrobial Susceptibility Testing (AST) machines can perform large numbers of MIC tests simultaneously for high-volume labs. Small microtitre trays (e.g., Sensititre) hold doubling dilutions of antimicrobials in media, instead of large Petri dishes used for the Kirby-Bauer manual method of sensitivity testing. Popular AST models are made by Becton-Dickinson, Micro-Media Systems Inc., and Anderson Technical Inc. Microtitre trays are reliable for all antimicrobial testing except metronidazole.

Becton-Dickinson's PASCO MIC machine is typical. It contains chromogenic and fluorogenic substrates. When bacteria touch the substrates, they have a positive or negative reaction that identifies their genus and species. PASCO tests the bacteria with increasing concentrations of antibiotic to determine if it has a susceptible, intermediate, or resistant phenotype. If a tray appears clear (no growth), subcultures are made from it to determine the Minimum Bactericidal Concentration (MBC) needed to kill 99.9% of pathogens.

## **Incubated culture**

Examining an incubated culture:

1. Visually assess the colony morphology on the agar plate (Plaques [motheaten]; R-type [rough]; S-type [smooth]).
2. Make a smear and check the shape of the pathogen under the microscope (coccus, flagellate, rod, spirilla, spirochete, and vibrio). Note the configuration (single micrococci; paired diplococci; clustered staphylococci; chained streptococci; sarcinae groups of eight). Note if they have flagella (None = atrichous; one end = monotrichous; both ends = amphitrichous; tuft = lophotrichous; all over = peritrichous).
3. Determine if the pathogen retains a stain in its outer wall or resists it.
4. Note if there is a mucoid capsule; if so, the bacteria are likely more virulent.
5. Note if spores are present and their position (central, subterminal, or terminal).
6. Note how they are reproducing (budding, branching, filaments, fission or conidia).
7. Use an atlas and fluorescent antibody assay, if available, to confirm what you see.

## **Concentrating a mycobacterial specimen**

Use a BL-3 room. Wear personal protective equipment (PPE) that includes a long-sleeved gown, gloves, and a respirator. Warm the sample at room temperature for 20 minutes. Dilute the sample 1:10 with Tris buffer or sterile, tissue culture-grade water. Coat the inside of a 15 mL centrifuge tube with 1 mL of 7H11, or LJ, or C-18 media. Transfer the sample in 5 mL transport media to the centrifuge tube. Add 4 mL of fresh media to the centrifuge tube. Shake at 140 rpm for 90 minutes at 37°C. Centrifuge for 20 minutes at 3,800 g and 30°C. Discard the supernatant. Resuspend the cells in 1 mL of sterile, filtered water. Add 5 mL of fresh media to a flask; incubate for 15 minutes at 37°C. Pour the centrifuge tube contents into the flask. Incubate at 37°C and check growth every two days. Autoclave all used materials before disposal.

## **Decontaminating a mycobacteria vial**

Make a *mycobacteria smear* in a BL-2 room. Wear a lab coat and gloves. Dip or spray the sample vial with 5% sodium hypochlorite for 15 minutes. Make the smear in a Class II Biological Safety Cabinet (BSC). Heat the smear 2 hours at 65°C—75°C to prevent aerosolization. Check for acid-fast bacilli (*AFB*) with acid-fast stain. If the smear and/or culture are positive, then confirm the identity of the isolate by *AccuProbe*, biochemical

analysis, or lipid profile on a gas-liquid chromatograph. Get a baseline *Mantoux test* for tuberculosis and a chest x-ray when you start working with mycobacteria. Get retested by Mantoux every 3 — 6 months, depending on the amount of exposure you have had. If you or your co-workers have a skin conversion, then retest every 3 months until no more conversions occur in your lab. You may ask your employer for BCG live vaccine.

### **Basic terms defined**

The following terms:

- *Bacteria*: No membraned nucleus (prokaryotic); primitive, single-celled, from 0.1  $\mu\text{m}$  to 10  $\mu\text{m}$ ; can be free-living, parasites (living on a live host), or saprophytic (living on decaying matter); reproduce quickly; waste products cause disease; cytoplasm center, covered by a cell wall made of protein and complex carbohydrates; transported by animals, plants, wind, and water.
- *Spores*: Dormant (sleeping) bacteria waiting for more hospitable growing conditions, or the seeds of algae, fungi, plants, and a few protozoans; not killed by sanitizing (washing) — only autoclaving instruments will kill spores.
- *Fungi*: Rigid cell wall; plant-like, single-celled, from 8  $\mu\text{m}$  to 10 mm; no green chlorophyll; include lichen, mildews, molds, mushrooms, rusts, smuts, and yeasts; decompose organic matter; absorb nutrients through hyphae and mycelium.
- *Protozoans*: Membraned nucleus (eukaryotic); single-celled animals that can be visible to the naked eye; flagella and cilia make them motile for hunting food; include amoeba, paramecia, and trypanosomes.

# Mycology

## Collecting mycology specimens

Remove bacteria from the infected area with an alcohol prep. Darken the room. Shine a Wood's Lamp over skin and hair to find lime green fluorescent areas of fungal infection. Scrape flaking skin into a sterile container with a tongue depressor. Tweeze out fluorescent hair. Gently scrape nail beds with a scalpel. Use aseptic technique to collect blood cultures. Use these media to perform mycology tests: Vitek yeast biochemical card; RapID yeast plus system; BactCard Candida test; KOH/Calcofluor fungal smear; Dermatophyte Test Medium (DTM); BACTEC media plate; SP-4 Diphasic broth. Keep specimens in subdued light at room temperature. Sterilize a loop in a Bunsen burner, and pass the mouths of the culture tubes into the flame three times. Keep the tubes parallel to the workbench to reduce contamination. When the loop cools, collect the specimen from the tube and streak the prepared media plate. Cover the plate and label it.

## Examining fungal specimens

Mix fungal scrapings with two drops of 10% potassium hydroxide (KOH) and one drop of Lacto Phenol Cotton Blue (LPCB) on a glass slide. Place a slide cover on top. Warm the slide in a Bunsen burner flame. Examine under high magnification to identify arthrospores, budding yeast and fungal fragments. Add a drop of Calcofluor white to a tissue specimen on a slide before heat fixing it. Examine the slide under bright-field or phase-contrast on the microscope. Infected tissue fluoresces white. To find cryptococcus capsules on a wet mount, dye the background with black India ink as a negative stain; the capsule remains clear. A fungal serology titer of more than 1:32 confirms your microscopy. Draw another titer three weeks later; if it increases four or more times, that strongly suggests fungal disease, but it may not find aspergillus or candida. Use latex serology for cryptococcal antigen to find meningitis, complement fixation for coccidiomycosis and histoplasmosis, and immunodiffusion for blastomycosis.

## Overgrowth of *Candida albicans*

*Candida albicans* (monilia) is a yeast that is usually a commensal part of the flora. It usually is one-celled and benign, but can develop filaments, become multicelled, and cause opportunistic infections. *Candida* overgrowth (candidiasis or moniliasis) is responsible for white thrush in the mouths of nursing babies and in the infected breasts of their mothers. It frequently causes vaginal yeast infections, where it resembles cottage cheese. *Candida albicans* causes systemic yeast infection (fungemia) in immunocompromised patients (e.g., AIDS, cancer, and transplant patients). Identify *candida albicans* with a wet mount: Swab the infected area and mix with saline on a glass slide. Add a cover slide. Examine it on low power. You will see budding yeast and hyphae.

Use Candida Isolation Agar to isolate *Candida albicans*. It contains malt and yeast extracts, peptone, and dextrose as nutrients, and aniline blue as a marker. Incubate at 30°C for 18—72 hours. *Candida albicans* will fluoresce yellow-green.

### **Microbiology legal reporting requirements**

Every microbiology laboratory report must include the patient's name and contact information, birthdate, collection date, gender, identification number, accession number, laboratory findings, date of positive findings, and doctor's name and contact information. You must report getting a requisition for these diseases to Public Health by phone, or within one hour of finding them independently: Anthrax; botulism; brucellosis; plague; tularemia; viral hemorrhagic agents (Crimean-Congo, Ebola, Lassa, and Marburg). You must follow up with a written report by e-mail or fax within one working day. You must report these diseases to Public Health by courier, e-mail, fax, or surface mail within one day: Chlamydia; cryptosporidiosis; diphtheria; E-coli O157:H7; encephalitis; hepatitis A or B; listeriosis; malaria; measles; rabies; salmonella; syphilis; TB; typhoid; and vibrio. Public Health requires TB and salmonella subcultures and malaria smears for confirmation.

## Parasitology

### **Ova, cysts, larva, and parasites**

Examine *blood smears* for eosinophilia (general indicator of parasitic infection). *Blood antigen* test for cryptosporidia, *E. histolytica*, and *Giardia* proteins, or string test for *Giardia*. Examine *sputum* for lung flukes, hookworm or hydatid cysts. *Bone marrow, CSF, lymph, or liver biopsy* may be required to find filaria, leishmaniasis, malaria, toxocara, toxoplasmosis, trypanosomes, or schistosomes. For all other parasites, collect three consecutive *stool* specimens in formalin. *Wet mount* fresh, liquid stool with LPCB stain to check for motile trophozoites. Strain well-formed stool. Concentrate it by centrifuging at 2000 rpm for 4 minutes in a conical tube. Ream the tube with a wooden stick. Add 10% formalin to make a tan suspension. The smear is thin enough if you can read a newspaper through the slide. Examine at 10X for parasites and 40X for blood. Mix stool with PVA plastic powder to glue it onto the slide before permanent staining with iodine or *Snap-N-Stain*. Use an ocular micrometer to measure parasites. Artifacts mistaken for parasites include bean sprouts, hairs, and peach fuzz.

### **Parasitology results**

Your lab must keep reference slides or a parasite atlas for comparison with diagnostic specimens. Check the stains and antigen detection kits you use with positive and negative controls every time you receive a new shipment and monthly thereafter. Use the right stain for the right specimen. Calibrate the ocular micrometer every time a new technician is hired, each time you change optics, and annually thereafter. Do not leave stools unrefrigerated more than 3 hours without fixing them with preservative. Report finding these parasites to Public Health: *Cryptosporidium parvum*, *Cyclospora cayetanensis*,

Entamoeba histolytica, Haematoxylin, Giardia duodenalis, Plasmodium falciparum, Taenia, Trichinella spiralis, Enterobius vermicularis. Give Public Health the referring doctor's contact information, so that they can correlate the patient's status and history. Keep positive specimens in the lab for at least one year, either as a permanently stained slide, or as a preserved stool sample that is safely stored. Public Health may order them for examination.

## Virology

### Dealing with specimens

Collect virology specimens within 4 days after symptoms appear. Waiting 7 or more days may lead to a false-negative result. Do not use bacterial collection equipment, like cotton swabs or wooden tongue depressors, because the viruses will die and the result will be false-negative. Swab or scrape the infected area with viral collection equipment for a direct sample, if possible, e.g., throat swab. If you cannot obtain direct samples because the site is inaccessible, like the central nervous system, then collect samples from different sites, like a blood viral titer, stool sample, throat swab, and urine because you need corroboration from at least two sources. Remember viruses are more fragile than bacteria, and lose infectivity quickly when removed from their host. Refrigerate viral samples immediately after collection to prevent overgrowth of bacteria and fungi. Transport them on ice to the lab as soon as possible. Freezing and thawing causes many viruses to die.

### Nonculture tests

Viral nonculture tests, like DFA, were developed in 1984 as a "results-while-you-wait" method of dealing with fragile viruses that cause collection and transport problems for the lab, and make the patient wait 5 days for diagnosis. Unfortunately, they are only 70% — 90% as accurate as culture results and usually require invasive collection techniques. Enzyme immunoassay (EIA) and nucleic acid probes (PACE 2) are semi-automated tests for high volume labs, created in the late 1980s, which can detect 10 to 100 organisms present. They are 98% specific, but only 75% — 80% sensitive for cervical viruses, less for male urethral swabs, and cannot be used on female urine at all. More recent, sensitive tests are polymerase chain reaction (PCR) and ligase chain reaction (LCR) to amplify DNA, and transcription mediated amplification (TMA) to amplify RNA, which can detect 1 to 10 organisms. Eventually PCR, LCR, and TMA may be sensitive enough to use on non-invasive samples, like urine.

### Isolating and identifying viruses

Homogenize a piece of tissue infected with virus by grinding it inside a biological safety cabinet (BSC). Centrifuge the specimen to remove large debris. Place the fine material onto a layer of chicken cells grown on media in a lab flask. Viral infection causes cells to form many nuclei, or create giant cells after several days. Spin the cells in an ultracentrifuge to separate them from their fluid, which retains the virus. Use a polymerase

chain reaction (PCR) to amplify the genetic material so the nucleic acid sequence can be analyzed for similarities to other viruses. Aliquot some fluid and another tissue sample for Histopathology, where the virus will be dyed green with an antibody/fluorescent marker stain and viewed with a transmission electron microscope (TEM) at 80,000X. Previously unclassified viruses are sent for animal antibody testing by ELISA; the media plate changes color if antibodies are present. SNT can also be used; if no antibody is present, the virus causes holes in the cell layer, giant cells, and multiple nuclei.

## Immunology

### **VDRL**

Venereal Disease Research Laboratory (VDRL) test is an old syphilis screening test, performed on blood or cerebrospinal fluid. It measures *Treponema pallidum* antibodies by flocculation reaction to the diphosphatidyl glycerol in ox heart extract -- fleecy bits of colloid precipitate out of the test solution when antigens react to cardiolipin antibodies. VDRL becomes positive three to four weeks after infection, so early cases are missed. VDRL is false-negative for half of all late-stage syphilis cases. False-positives can occur if the patient has hepatitis, HIV, leprosy, lupus (SLE), Lyme disease, malaria, mononucleosis, pneumonia, rheumatic fever, or rheumatoid arthritis because the antibodies produced are all similar. Perform an ELISA test to differentiate the antibodies and confirm syphilis infection. Perform another VDRL to measure the patient's response to treatment. If the titer decreases, then treatment is working well. If the titer increases, then the patient has been reinfected, or the treatment is failing. Six months of treatment are necessary for the titer to become negative. VDRL is also sometimes false-positive in pregnancy.

### **FTA-ABS**

Fluorescent *Treponema pallidum* Antibody Absorption (FTA-ABS) test is a specific blood test for syphilis. Do not use CSF. It is more expensive than VDRL or RPR, so use it for confirmation, not screening. It is more accurate than VDRL because nonspecific antispirochetal antibodies are removed from the blood by dilution. Mix the diluted serum with Nichol's strain of *Treponema pallidum* on a glass slide. Syphilis antibodies bind to the antigen and can be found with fluoresceinated antihuman gamma-globulin antibody. False-negatives occur in early or late syphilis, but FTA-ABS is 100% accurate for secondary syphilis. Two percent of test subjects have borderline results that require follow-up with *Treponema pallidum* Antibodies blood test (TP-PA) or an MHA-PA microhemagglutination test. False-positives occur with ANA, bejel, cold agglutinins, herpes, SLE, leprosy, Lyme disease, malaria, pinta, relapsing fever, or yaws. Do not use FTA-ABS, MHA-PA, or TP-PA to monitor treatment, because the patient will always have a positive result after contracting syphilis even once.



## Newer flocculation tests

Plasmacrit test (PCT) is a syphilis screening method that uses less blood than VDRL. It can be performed with a finger prick, rather than a venipuncture. Fill a heparinized hematocrit tube with capillary blood. Centrifuge it, then mix the plasma with 0.01 mL cardiolipin antigen anti-inhibited with choline chloride. Agitate the mixture on a rocker for four minutes. If flocculation occurs, the result is positive for syphilis.

Rapid Plasma Reagin test (RPR) is used to screen for syphilis and monitor the effectiveness of treatment. If the antibiotics are working, the patient's antibody levels decrease. If antibody levels are constant or rise, either the treatment is ineffective, or the patient got reinfected. False-negatives are found in primary and tertiary patients. RPR can be confused by similar antibodies produced by HIV, Lyme disease, lupus, malaria, and pneumonia. If RPR is positive, follow up with a confirmatory FTA-ABS test or a Captia Syphilis EIA.

## Testing for mononucleosis

Epstein-Barr virus (EBV) causes mononucleosis. Confirm early infection (2—9 weeks) by Monospot heterophile antibodies test. Confirm later infection by EBV antibody test. Order with CBC and throat swab.

- *Monospot*: Drop capillary or venous blood on a glass slide. Mix with guinea pig kidney antigen to absorb Forssman antibodies. Mix with beef red blood stroma to absorb nonForssman antibodies. Mix with horse blood. Guinea pig agglutination means the patient has early mononucleosis. Beef should not agglutinate. Monospot can be false-negative on children under 10, or before two weeks of infection. Monospot may not detect infection older than six months. False-positives are caused by adenovirus, Burkitt's and Hodgkin's lymphomas, cytomegalovirus, hepatitis, HIV, leukemia, lupus, pregnancy, rheumatoid arthritis, rubella, and toxoplasmosis.
- *EBV antibody titer*: Serially dilute blood serum or other body fluids with saline. Negative is less than 1:40 and no IgM antibodies are present. Positive is greater than 1:40 and antibodies are present. IgM indicates the active phase of mono. IgG antibodies mean the patient is recovering from mononucleosis.

## Qualitative agglutination assay

Qualitative agglutination assays: Quick latex fixation tests (15—60 minutes) to find over 100 types of antibodies and antigens in blood, CSF, saliva, and urine. Since 1956, latex fix has been a first-line screening method, but it misses low reactivity and is confused by similar diseases. Mix body fluid with latex beads impregnated with a specific antigen or antibody on a glass slide. If the antigen or antibody is also in the body fluid, the latex clumps together (snows). You may need to absorb similar antibodies with guinea pig and beef blood to get an accurate result. Some latex agglutination tests are read with the naked eye. Some require spectrophotometers or nephelometers to read fine agglutination.

Common latex agglutination tests: Anti-streptolysin O (ASO); candida; ELISA for C-reactive protein (CRP); Haemophilus influenza type B; histoplasmosis; HSV2 IgG for herpes; Katex for Leishmaniasis; lupus; methicillin-resistant staphylococcus aureus (MRSA); mononucleosis; mycoplasma pneumoniae; Pastorex aspergillus for transplant patients; Recombigen for HIV-1; red cell agglutination (RCA) for Hepatitis B; rheumatoid factor; rotavirus; TV for Trichomonas vaginalis.

## **Important terms**

*AAC*: Antibiotic-associated colitis from *C. difficile*.

*BHIB*: Amber pig brain and heart media to cultivate fastidious organisms in food and water. Add 0.1% agar to lower the oxygen tension for aerobic, microaerophilic, and obligate anaerobes.

*Chilomastix mesneli*: Intestinal parasitic protozoan with flagellae, transmitted through the fecal-oral route. It has lemon-shaped cysts and one nucleus.

*Commensal avirulent*: Humans are an ecological niche for the life form; it does not help or hurt.

*Differential identification*: Naming bacteria according to the headspace gases and volatile compounds they release as they grow on media, with a spectrometer (microDMx).

*Giardia lamblia*: Parasite of the small intestine transmitted through the fecal-oral route and fresh water that causes traveler's diarrhea. Cysts are activated by stomach acid and become trophozoites. Tape a gelatin-covered string to the cheek, swallow the other end into the duodenum, leave it several hours, then examine the fluid microscopically. Cure with a week-long course of Flagyl.

*Growth curve*: Lag phase micro-organisms grow slowly while adapting to the shock of being taken from the host and put on media. Log phase micro-organisms double every few minutes. Stationary phase micro-organism numbers plateau because they compete for scarce food. Death phase micro-organisms outstrip their food supply and excrete so much waste that the colony dies off.

*Growth factor*: Serum protein binds to a receptor on a cell's surface, encouraging the cell to split into daughter cells.

*HACEK*: Acronym for Haemophilus, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corodens, and Kingella kingae. They are commensal flora in the mouth and throat, gram-negative, fastidious, and grow slowly. Overgrowth causes conjunctivitis, bone and dental disease, endocarditis, middle ear infections and peritonitis. HACEKs are susceptible to cephalosporins.

*Host resistance:* The patient resists disease, either by innate immunity that works against any invader (e.g., cilia, inflammation, phagocytosis), or adaptive immunity that works against specific invaders (antigen/antibody reaction).

*Immunologic method:* Identifying pathogens with antigen/antibody markers.

*Jarish-Herxheimer reaction:* A temporary flare side-effect of antibiotic treatment. As pathogens die, they release endotoxins that give the patient chills and fever, headache, muscle cramps, and skin lesions. Patients with rheumatoid arthritis are particularly susceptible to Jarish-Herxheimer flares.

*Molecular method:* Identifying an unclassified pathogen by amplifying its genetic material with a polymerase chain reaction (PCR), analyzing the nucleic acid sequence, and comparing it to known pathogens.

*Numeric taxonomy:* Also called phenetics. It is a bacterial classification system created by Michael Adanson. It ranks micro-organisms according to how similar they are genetically and morphologically. Bacteria that are closely related are called a cluster. Its aim is for the classification into taxa to be objective and repeatable.

*Package kits:* For quick screening when there is no time for a culture. They contain PCR to amplify the genetic material, and then the 1450 base pair region of the 16S rDNA gene can be sequenced by electrophoresis. SWOrRD, TaqMan, and MicroSeq are examples of quick screening kits. They are not as accurate as cultures.

*Pathogenicity:* The ability of a pathogen to multiply in a host and cause a disease that hurts its host.

*PBP:* Penicillin-binding protein, which makes a bacterium's wall resistant to penicillin and all other b-lactam antibiotics. If there is no PBP, when an antibiotic is introduced to the bacterium it lengthens, develops holes, and lyses. If there is PBP, the bacterium stays intact.

*Presumptive identification:* Naming bacteria by looking at their size and shape under the microscope, and the colony morphology on media. Note the color, outline (circular, rhizoid, or wavy), elevation (convex, flat, or raised), and translucency (opaque, translucent, or transparent).

*Thayer-Martin agar:* Chocolate-brown blood agar cooked at 56°C until the cells lyse. Use for fastidious organisms like *Neisseria gonorrhoea* and *Haemophilus influenzae*.

*Trichomonas hominis:* Harmless protozoan with a pyriform body, undulating membrane, and a lancet-shaped nucleus with granules.

*Trichomonas vaginalis*: Oval parasite with flagellae and corkscrew motility on wet mount that causes vaginal infections with greenish, foul-smelling froth and can cause premature births.

*Virulence*: The degree of pathogenicity (ability of a pathogen to cause disease), and the severity of that disease.

# Practice Test

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## *Practice Questions*

1. Receiving cannot accept a specimen unless it has:
  - a. A correct, legible label
  - b. An uncontaminated, signed requisition with billing information
  - c. An intact container with correct media
  - d. All of the above
  
2. A laboratory refrigerator used to store volatile, flammable liquids can hold:
  - a. 120 gallons of class I, II, and IIIA liquids
  - b. 180 gallons of class I, II, and IIIA liquids
  - c. 200 gallons of class I, II, and IIIA liquids
  - d. 50 gallons of class I, II, and IIIA liquids
  
3. Disease incidence predicts:
  - a. How probable it is a patient will develop a disease, and its etiology
  - b. How likely a test result is to be right or wrong, given certain variables
  - c. How likely the patient with a negative test really does not have the condition
  - d. How likely the patient with a positive test result really has the condition
  
4. Beer's law in spectrophotometry
  - a. Means a transparent sample transmits 0% light
  - b. Only applies if absorbance is between 0.1 and 1.0
  - c. Means an opaque sample transmits 100% light
  - d. Uses a visible spectrum from 340 nm to 500 nm
  
5. Naming bacteria by looking at their size and shape under the microscope, and the colony morphology on media is:
  - a. Differential identification
  - b. Numeric taxonomy
  - c. Presumptive identification
  - d. *TaqMan* electrophoresis
  
6. OSHA allows ungloved phlebotomy only if:
  - a. The experienced phlebotomist working in a volunteer blood donor center agrees.
  - b. The phlebotomist's skin is intact and the patient is cooperative.
  - c. Both a and b
  - d. The patient is an infant.

7. The hospital department that studies alcohol, drugs, poisons, and heavy metals is:
  - a. Serology/Immunology
  - b. Toxicology
  - c. Cytology
  - d. Endocrinology
  
8. A hemoglobin electrophoresis result of adult hemoglobin (HbA) or HbA<sub>2</sub> means the patient has:
  - a. Sickle cell anemia
  - b. Fetal hemoglobin
  - c. Normal hemoglobin
  - d. Hemolytic anemia
  
9. US law overrides the patient's right to confidentiality if:
  - a. The patient has a sexually transmitted disease or tuberculosis (TB)
  - b. The caregiver is likely to be infected
  - c. Authorities suspect child abuse or neglect under CAPTA
  - d. All of the above
  
10. The recall rate is also known as the:
  - a. Sensitivity
  - b. Specificity
  - c. Aliquot
  - d. Circadian rhythm
  
11. Biochemistry usually requires:
  - a. Lavender, light blue, and black blood collection tubes
  - b. Red, pink, and yellow blood collection tubes
  - c. Green, gray, and marbled serum-separator tube (SST) blood collection tubes
  - d. Navy, purple, and brown blood collection tubes
  
12. A normal kidney function study shows a:
  - a. BUN to creatinine ratio between 15:1 and 20:1
  - b. Alkaline phosphatase 30 to 85 international milliunits/mL
  - c. Serum aspartate aminotransferase 5 to 40 international units/L
  - d. Amylase 56 to 190 international units/L
  
13. A newborn's jaundice could be caused by:
  - a. Erythroblastosis fetalis
  - b. Kernicterus
  - c. Physiologic jaundice from poor fluid intake
  - d. All of the above

14. Lipids from carbohydrate and alcohol sources are:
- Anions
  - Triglycerides
  - Cholesterol
  - Eluent
15. When serum proteins indicates disease, the doctor usually follows up with:
- Total protein, albumin, and globulin
  - Ascites
  - Protein electrophoresis
  - Bilirubin
16. Elevated creatine phosphokinase (CPK) could mean myocardial infarction, but could also mean:
- Alcoholism, hypothyroidism, cardioversion, or clofibrate use
  - Aspirin, burns, warfarin, or sickle cell anemia
  - Lung disease or congestive heart failure
  - Crushing injury, bowel infarction, or opiate use
17. A patient whose cortisol level is high at both 8:00 a.m. and 4:00 p.m. likely has:
- Addison disease
  - Natriuretic factor
  - Diabetes insipidus
  - Cushing syndrome
18. Decreased sodium in the blood is:
- Hypernatremia, often from diabetes, burns, or Cushing syndrome
  - Hyponatremia, often from vomiting and diarrhea, furosemide, or Addison disease
  - Hyperkalemia, often from acidosis, spironolactone, or kidney failure
  - Hypokalemia, often from alkalosis, stomach cancer, or eating too much licorice
19. CPK in a patient with a myocardial infarction will:
- Rise 6 hours after heart attack, peak in 18 hours, and return to baseline in 3 days
  - Rise 6 to 10 hours after heart attack, peak at 12 to 48 hours, and return to baseline in 4 days
  - Rise 24 to 72 hours after heart attack, peak in 4 days, and return to baseline in 14 days
  - Cause a corresponding rise in alpha-fetoprotein
20. The panic value for blood pH is:
- 7.35
  - Less than 7.20
  - 80 to 100 torr
  - 4.0 to 8.0 mcg/L

21. The liver destroys old blood cells at the end of their lifespan of:
- 120 days
  - 30 days
  - 1 week
  - 90 days
22. An erythrocyte sedimentation rate (ESR) measures how blood cells with anticoagulant aggregate in a Westergren or Wintrobe tube in 1 hour because of changes in plasma proteins, which is known as :
- Retic count
  - Serendipity
  - Fibrinolysis
  - Rouleaux formation
23. If the doctor suspects the patient has Hodgkin disease, then the correct stain for the smear is:
- Periodic acid-Schiff (PAS)
  - Sudan black B (SBB)
  - Leukocyte alkaline phosphatase (LAP)
  - Lactophenol cotton blue (LPCB)
24. A battlement scan is preferable to a wedge scan for studying bone marrow because:
- Battlement technique distributes cells evenly across the slide
  - Lymphocytes concentrate in the feather
  - Wedge technique causes leukocytes to pool in different sections of the slide
  - Both a and c
25. Most coagulation (clotting) disorders are due to:
- Phase I problems
  - Factor VIII deficiency
  - Fibrinolysis
  - Factor III distress call from the injury site
26. If the patient's PT and PTT are longer than 70 seconds, then check if \_\_\_\_\_ caused a false result:
- Ferritin
  - Lupus inhibitor antibody (LA)
  - Platelet antibody
  - Intrinsic factor
27. Confirm a fungal infection found through microscopy with a:
- Latex serology for cryptococcal antigen
  - Fungal serology titer of more than 1:32 that increases x4 or more 3 weeks later
  - Complement fixation for coccidiomycosis and histoplasmosis
  - Immunodiffusion for blastomycosis.



28. Two modern flocculation tests that replace the older Venereal Disease Research Laboratory (VDRL) test for syphilis screening are:
- Plasmacrit test (PCT) and rapid plasma reagin (RPR) test
  - Fluorescent treponemal antibody absorption (FTA-ABS) and enzyme-linked immunosorbent assay (ELISA)
  - Treponemal-specific microhemagglutination (MHA-TP) and *T. pallidum* particle agglutination test (TP-PA)
  - Captia Syphilis-G enzyme immunoassay (EIA) and cold agglutinins
29. To make a dilution of  $\frac{1}{2}$  or 1:2
- Dilute  $\frac{1}{2}$  mL of serum with 2 mL of saline
  - Dilute 1 mL of serum with 2 mL of saline
  - Test undiluted serum for antibody/antigen reaction against a control
  - Dilute 1 mL of serum with 1 mL of saline
30. A Monospot test uses ingredients from:
- Guinea pig, cow, and horse
  - Sheep, pig, and horse
  - Dog, sheep, and rabbit
  - Fish, cat, and ferret
31. A prozone phenomenon occurs when performing an antibody titer on a patient with:
- Epstein-Barr virus (EBV)
  - Reynaud disease
  - Both syphilis and HIV
  - Immunoglobulin G (IgG) antibodies
32. An Rh- mother who is pregnant with the child of an Rh+ father needs Rh immunoglobulin (*RhoGAM*):
- Even if the pregnancy ends in miscarriage or abortion
  - At 26 to 28 weeks of pregnancy and again within 72 hours after her delivery.
  - During her labor
  - Both a and b
33. If your patient has a mild transfusion reaction:
- Eosinophilia, hypocalcemia, leukopenia, and pancytopenia may occur
  - Dyscrasia, leukocytosis, hypercalcemia, and leukemia may occur
  - Anemia, hypokalemia, glycosuria, and pancytopenia may occur
  - Hemolysis, hyperkalemia, hypoglycemia, and hemoglobinuria may occur
34. Type O blood has:
- B antigen and anti-A antibody
  - A antigen and anti-B antibody
  - No A or B antigens and no anti-A or anti-B antibodies
  - No A or B antigens and no anti-A or anti-B antibodies

35. Choose the top priority transfusion patient from the list below.
- Cardiac surgery patient who lost more than 1,200 mL of blood
  - Trauma patient with a hemoglobin of 5 g/dL
  - Pernicious anemia patient
  - Hemophiliac boy at regular clinic visit
36. Reject a transfusion request when:
- Recipient blood specimen is hemolyzed
  - The patient armband does not have a unique identifier
  - Donor blood is lipemic, clotted, or contains foreign objects
  - All of the above
37. To diagnose a urinary tract infection correctly, the microbiology lab requires a:
- Midstream urine collection (MSU)
  - Witnessed urine collection
  - 24-hour urine collection
  - Random urine collection
38. When assisting the doctor with cerebrospinal fluid (CSF) collection, you need 4 tubes for:
- Cell count, glucose and protein, gram stain and culture, virology/mycology/cytology.
  - Immunoelectrophoresis
  - Fungus, oncology, and SMA-12
  - Neutrophilia, lymphocytophilia, glutamine, and lactate dehydrogenase (LDH)
39. Fusobacteria cause:
- Botulism and *Listeria* infections
  - Lyme disease and *Helicobacter pylori* stomach ulcers
  - Pyorrhea and Lemierre syndrome
  - Chlamydia genital infections and pneumonia
40. The type of media required to incubate a TB culture correctly is:
- Tinsdale
  - Sheep blood agar
  - Modified Wadowsky-Yee (MWY)
  - Löwenstein-Jensen (LJ) egg
41. To find parasites under the microscope, set the magnification to:
- 40x
  - 10x
  - 1000x
  - 400x

42. Identify the parasite that must be reported to Public Health authorities:
- Crypto (*Cryptosporidium parvum*)
  - Hookworm (*Ancylostoma duodenale*)
  - Tapeworm (Cestoda)
  - Pinworm (*Enterobius*)
43. To identify motile trophozoites:
- Examine blood smears and blood antigens
  - Perform a string test
  - Use *Snap n' Stain* on sputum
  - Wet mount fresh, liquid stool with LPCB stain
44. Public Health requires you to keep positive parasitology samples preserved for :
- The patient's lifetime
  - One year
  - Ten years
  - One month
45. Shine a Wood's lamp over the patient's skin to help you collect:
- Malaria specimens
  - Public Health specimens
  - Toxicology specimens
  - Mycology specimens
46. Identify the positive dipstick test that would indicate an *E. coli* infection:
- Leukocytes
  - Protein
  - Ketones
  - Nitrites
47. A pregnancy test may be ordered for a man with:
- Testicular cancer
  - Prostatitis
  - Cryptorchidism
  - Peyronie disease
48. Normal urinary output for a 24-hour urine test is:
- 4 quarts
  - 150 to 500 mL
  - 30 liters
  - 750 to 2,000 mL

49. Urate crystals found during microscopic urinalysis indicate:
- a. Urea-splitting bacteria are present
  - b. Poisoning
  - c. Gout
  - d. Hyperparathyroidism
50. Patients with polycystic kidney disease (PKD) often have:
- a. Cirrhosis and hepatitis
  - b. Coronary artery bypass graft (CABG) and hypertension
  - c. Hematuria and uroliths
  - d. Melena and hematochezia

## Answers and Explanations

- D: All of the above. Receiving cannot accession a specimen without:

  - A label that clearly states the patient's name, collection date, doctor's name and contact information, specimen type, and test required.
  - An uncontaminated, valid requisition bearing the doctor's signature, patient's billing information, and pertinent information (acute or convalescent phase, antibiotic use, fever, or traveler).
  - Intact specimen container.
  - Correct media type or preservative used for the specimen type.
  - Same-day collection date, or preincubated at room temperature or subcultivated, and then vented, to prevent false-negatives of nonfermentative species.
  - If the specimen does not meet these conditions, call the doctor's office and get the missing information. Discard the specimen if you cannot obtain full information, and inform the doctor's office that recollection is required.
- A: 120 gallons of class I, II, and IIIA liquids. Choice A complies with fire safety standards. Class IA, IB, and IC are flammables. Class II, IIIA, and IIIB are combustibles. No more than 120 gallons of class I, II, and IIIA liquids can be stored in a lab fridge, and of those, no more than 60 gallons may be class I and II. Do not locate more than three storage cabinets in one fire area. No more than 50% of the flammables can be stored for teaching. Use DOT-approved glass, metal, or polyethylene containers no larger than 1.1 gallons (4 L).
- A: How probable it is a patient will develop a disease, and its etiology. A is correct because disease incidence measures how prevalent a disease is among a given population in a specific place, over a specific time. Incidence predicts how probable it is a patient will develop a disease, and its etiology (likely cause). B, C, and D are incorrect because they refer to a related concept called predicted values, which estimate how likely a test result is to be right or wrong, given certain variables, such as the patient's age, occupation, race, income, how long the symptoms have lasted, and if there is fever.
- B: Only applies if absorbance is between 0.1 and 1.0. B is correct because Beer's law states absorbance is proportional to the concentration of a solution, but Beer's law only applies if absorbance is between 0.1 and 1.0. Different substances absorb different light wavelengths, so a spectrophotometer (Spec-20) compares the intensity of light entering a sample and exiting from it (percent transmittance) to find the concentration of the sample. A completely transparent sample has 100% transmittance. A completely opaque sample has 0% transmittance. Visible spectrum light ranges from 440 nm to 700 nm.
- C: Presumptive identification. C is correct. Note the color, outline (circular, rhizoid, or wavy), elevation (convex, flat, or raised), and translucency (opaque, translucent, or transparent) for presumptive identification. Differential identification means naming

bacteria according to their headspace gases and volatile compounds they release as they grow on media, with a spectrometer (microDMx). Adanson's numerical taxonomy (phonetics) ranks microorganisms according to how similar they are genetically and morphologically. Closely related bacteria form a cluster, which is classified into objective, repeatable taxa. *TaqMan*, *SWOrRD*, and *MicroSeq* are quick screening kits. They are not as accurate as cultures but are quicker when time is critical. Polymerase chain reaction (PCR) in quick kits amplifies the genetic material, and then the 1450 base pair region of the 16S rDNA gene is sequenced by electrophoresis.

6. C: Both a and b. The US Occupational Safety and Health Administration (OSHA) sets the standards for proper hand washing, wearing gloves, bagging specimens in biohazard bags, and disposing of needles and lancets in a sharps safe container. Visit <http://www.osha.gov> regularly to find updates. OSHA requires phlebotomists to glove before every venipuncture as part of standard (universal) precautions for bloodborne pathogens. Only in a volunteer blood donation center does OSHA allow for ungloved venipunctures, providing the phlebotomist is experienced and not a trainee. The phlebotomist must agree not to glove. The employer must provide gloves if the phlebotomist wants them. If the phlebotomist is allergic to latex or powdered gloves, the employer must provide hypoallergenic or unpowdered gloves, liners, or barrier cream. The phlebotomist must glove if her skin is broken or burnt, or has a rash. If the patient is combative or likely to have a reaction, then the phlebotomist must glove. Employers must reevaluate the no-glove policy periodically to see if it continues to be a low-risk option. Whenever contact with blood is likely, such as during infant heel pricks, gloves are required.
7. B: Toxicology. Serology/Immunology studies antibodies in the liquid part of blood. Cytology studies cells for cancer, such as Pap smears. Endocrinology studies hormones, such as diabetes and acromegaly.
8. C: Normal hemoglobin. Hemoglobin electrophoresis differentiates hemoglobin into normal HbA and normal HbA<sub>2</sub>, or abnormal HbS in sickle cell patients, or HbC in hemolytic anemia patients, or HbF in a fetus or newborn.
9. D: All of the above. Doctors, nurses, social workers, chiropractors, law officers, daycare staff, clergy, teachers, and psychologists were declared mandatory reporters in 1996. This means they must report certain occurrences or suspicions orally to the proper authorities within 24 hours and follow up with a written report within 48 hours. For example, a doctor must report STD or TB to Public Health to prevent an epidemic. Caregivers have the right to know the patient's diagnosis if it puts them at risk for infection or assault. Suspicion of child abuse, exploitation, or neglect is reported to Child Protective Services under the Child Abuse Prevention and Treatment Act (CAPTA). Each state has an abuse hotline. Many states require *anyone* who has reasonable cause to report child or elder abuse or face civil liability. You must know the law of the state in which you practice.

10. A: Sensitivity. Sensitivity (recall rate) measures how many times a test produces true-positive results, which indicates patients probably have a disease, compared with the gold standard test for that particular illness. Sensitivity allows early detection of disease and prevents epidemics. Divide the number of patients who definitely have the disease and test positive by the total patients tested who have the disease (including those who tested false-negative), and multiply by 100 to obtain the percentage sensitivity. Specificity measures how many times a test produces true-negative results, meaning patients probably do not have a disease, compared with the gold standard test for that particular illness. Specificity is important for cancer chemotherapy and other toxic treatments. Aliquot is dividing a solution into equal parts. Aliquot allows very expensive reagents or drugs, and blood samples that are below scale, to be used efficiently. Circadian rhythm is a normal daily flow that affects hormones, which are normally higher in the morning than in the afternoon.
11. C: Green, gray, and marbled SST blood collection tubes Hematology requires mostly lavender, light blue, and black tubes. Blood Bank and Public Health require red, pink, and yellow tubes. Toxicology requires navy, purple, and brown tubes. If you draw the wrong color tube, it contains an inappropriate anticoagulant, and the test will be invalidated.
12. A: BUN to Creatinine ratio between 15:1 and 20:1. Blood urea nitrogen (BUN) and creatinine are waste products of protein metabolism, measured in kidney function tests performed with a 24-hour urine. If the kidneys do not filter properly, creatinine output in the urine decreases, and creatine blood levels increase. High creatinine (more than 1.5 mg/dL) and BUN (more than 20 mg/dL) means the patient has a kidney disease (e.g., glomerulonephritis, pyelonephritis, stones, tubular necrosis, tumors). BUN and creatinine must be in correct proportion for optimal health. ALP and AST are liver function tests. Amylase is a pancreas test.
13. D: All of the above. Newborn jaundice is different from adult jaundice. Babies have more red blood cells and reticulocytes than adults do. Babies have immature livers that are not yet efficient at breaking down bilirubin. Adult jaundice is usually from hepatitis or cirrhosis of the liver. Erythroblastosis fetalis means the baby's Rh factor is incompatible with his mother's Rh, leading to hemolytic disease of the newborn. Kernicterus means the bilirubin is greater than 5 mg/dL, resulting in hemolytic anemia if not treated with phototherapy (blue lights). Physiologic jaundice occurs in breastfed babies released from the hospital too early and without a vitamin K injection, but resolves in a week with adequate fluids.
14. B: Triglycerides. Anions are negatively charged ions of potassium and sodium. Cholesterol is lipids from animal sources that climb after a fatty meal. An eluent is a solvent used for chromatography.
15. C: Protein electrophoresis. The serum proteins test includes total protein, albumin, and globulin. Ascites is swelling of the abdomen from extra fluid in the peritoneum, resulting from end-stage diseases of the heart, kidney, liver, ovary, and pancreas. When

serum proteins make the doctor suspect one of these diseases, the doctor follows up with protein electrophoresis. Four globulin fractionations are added to the total protein and albumin: alpha-1 globulin, alpha-2 globulin, beta globulin, and gamma globulin. Electrophoresis patterns and the patient's history of drug use help pinpoint the diagnosis, which may extend to rheumatoid arthritis, muscle tumors, and immune deficiencies. Bilirubin is the brownish-red bile pigment from broken down blood cells in the liver.

16. A: Alcoholism, hypothyroidism, cardioversion, or clofibrate use. Cardiac enzymes elevate soon after a heart attack, but that is not the only possible root cause. CPK elevates in alcoholism; cardiac catheterization; stroke; clofibrate use; electric shock applied during resuscitation; low thyroid hormone and high thyroid-stimulating hormone; and after surgery. B and D refer to situations that cause AST enzyme to rise. C refers to situations that cause LDH enzyme to rise.
17. D: Cushing syndrome. Cortisol is an adrenal stress hormone that is normally higher around 8:00 in the morning (6 to 28 mcg/dL) and lower at 4:00 in the afternoon (2 to 12 mcg/dL). The fluctuation is a normal diurnal variation. Cushing syndrome patients have sustained high cortisol. Addison disease patients have chronically low cortisol levels, diagnosed by a 24-hour urine test for 17-hydroxycorticosteroids. Abnormal cortisol levels also appear in thyroid and pituitary gland disease, obesity, and cancer, and when steroids, diuretics, or birth control pills are used, but it is not the same pattern as Cushing syndrome. B refers to atrial natriuretic factor (ANF), produced by the heart's atria during volume overload and high blood pressure.
18. B: Hyponatremia, often from vomiting and diarrhea, furosemide, or Addison disease. Hyponatremia results from too much water and not enough salt in the bloodstream. Hyponatremia often presents as a urine sample with a specific gravity (SG) lower than the normal 1.015 to 1.025 and closer to the SG of water (1.000). Hypernatremia refers to too much salt in the bloodstream, which increases SG above 1.025. Hyperkalemia and hypokalemia refer to the level of potassium, not sodium.
19. A: Rise 6 hours after heart attack, peak in 18 hours, and return to baseline in 3 days. CPK is the first enzyme to rise following a heart attack, so doctors measure it before the other cardiac enzymes. If creatine kinase-MB (CK-MB) rises, it means the heart sustained severe damage. B. refers to the response of AST to a heart attack. C refers to the response of LDH to a heart attack. D does not apply because alpha-fetoprotein (AFP) is used to find liver disease, testicular cancer, and birth defects.
20. B: Less than 7.20. pH stands for percentage of hydrogen. A blood pH test is performed with arterial blood gasses to determine if the patient has acidosis or alkalosis. The blood must be kept in a narrow range of pH from 7.35 to 7.45, so answer A would be low normal. Answer C, 80 to 100 torr, refers to normal percentage of oxygen. D is incorrect because an abnormal PSA result for prostate cancer is unrelated to blood pH.
21. A: 120 days. A normal erythrocyte (red blood cell [RBC]) lives for four months. When it wears out, the spleen destroys it and the liver converts it to bilirubin, which the



gallbladder stores as bile and the digestive system uses to break down dietary fats. Old red blood cells puff into a sphere, rather than retaining their original barbell-shape. Changing shape makes them fragile and inflexible. Babies have 4.8 to 7.2 million RBCs per cubic millimeter of blood. Adult males have 4 to 6 million, and adult females 4 to 5 million RBCs. Pregnant women have lower RBC counts.

22. D: Rouleaux formation. Normal red blood cells settle slowly when standing in a test tube: males 0 to 15 millimeters per hour; females 0 to 20 mm/h; children 0 to 10 mm/h; elderly patients 5 to 10 mm/h more. A low sedimentation rate indicates hemorrhage. A high sedimentation rate indicates cells are heavier and falling quicker than normal, usually from inflammatory diseases, parasite infection, cell death, heavy metal poisoning, or toxemia of pregnancy. However, cold agglutinins also make red blood cells adhere to each other in a Rouleaux formation when they are chilled.
23. C: Leukocyte alkaline phosphatase (LAP). Hematologists use LAP stain to highlight neutrophils when the patient has many white blood cells but not leukemia (leukemic reaction). Microbiologists use periodic acid-Schiff (PAS) to stain carbohydrates, collagen, fibrin, and mucin purple. Sudan black B (SBB) is specifically for acute leukemia patients; it helps to differentiate between immature cells by staining lipids in myeloid leukemia that are absent in lymphoid leukemia. LPCB is mixed with 10% potassium hydroxide (KOH) to identify fungus.
24. D: Both a and c. Make a bone marrow slide with a battlement technique so the review is more standardized, with even cell distribution. Wedge push technique (feathered end) causes the white cells to pool unevenly on the slide. On the side edges and in the feather of a wedge push slide, you will find concentrated pockets of eosinophils, monocytes, and segmented neutrophils. Small lymphocytes concentrate in the center of the slide.
25. A: Phase I problems. Phase I of coagulation happens in the first 3 to 5 minutes after an injury, when the platelets mobilize. Factor III distress call is sent in phase II, not phase I. Factor VIII deficiency is the problem that causes hemophilia. Fibrinolysis occurs when the injured site is plugged with a blood clot and plasminogen changes to plasmin.
26. B: Lupus inhibitor antibody (LA). Hematologists use phospholipid in PT and PTT tests to check how fast a patient clots. The normal range for PTT is 60 to 70 seconds. Lupus inhibitor antibody (LA) acts against the phospholipid and falsely extends clotting time. LA is implicated in miscarriages, rheumatoid arthritis, lupus, Reynaud syndrome, and thromboembolism. Platelet antibody testing is only appropriate if the patient has purpura and hemoglobinuria. Intrinsic factor is incorrect; it is the stomach's ability to produce B<sub>12</sub> to prevent pernicious anemia.
27. B: Fungal serology titer of more than 1:32 that increases x4 or more 3 weeks later. First, gently scrape suspected fungus off the patient's skin. Mix two drops of 10% potassium hydroxide (KOH) and one drop of LPCB on a glass slide, cover it, and warm it to observe budding yeasts. Add a drop of calcofluor white before warming to see fluorescent infected tissue. Put a drop of India ink on a wet mount to see clear

cryptococcal capsules. Confirm the microscopic exam with fungal serology when you test the skin scraping and again in three weeks. The doctor may follow up by ordering latex serology for cryptococcal antigen to find meningitis, complement fixation for coccidiomycosis and histoplasmosis, and immunodiffusion for blastomycosis.

28. A: Plasmacrit test (PCT) and rapid plasma reagin (RPR) test. The old screening test for syphilis is VDRL, which measures *Treponema pallidum* antibodies by flocculation reaction to the diphosphatidyl glycerol in ox heart extract. However, VDRL misses cases of syphilis that are less than four weeks old, and half of cases that are in the late stages. VDRL is not very sensitive, and often gives a false-positive result for patients with the following conditions: pregnancy, hepatitis, HIV, leprosy, lupus (SLE), Lyme disease, malaria, mononucleosis, pneumonia, rheumatic fever, or rheumatoid arthritis. PCT and RCR are less likely to be confounded, and since they require less blood, are replacing VDRL. ELISA confirms syphilis infection by identifying the specific antibodies. FTA-ABS is 100% accurate for secondary syphilis, but it is expensive, and the patient will always test positive once infected. Captia is required to confirm RPR. Cold agglutinins increase in children with congenital syphilis.

29. D: Dilute 1 mL of serum with 1 mL of saline. You must know how to dilute to perform a titer, which measures how many times a blood sample must be diluted with saline before an antibody can no longer be found in it.

First, check the antibody/antigen reaction against the controls with undiluted serum. To prevent blood clotting (Rouleaux formation) during dilution, warm the blood and saline to body temperature (37°C) for 10 minutes before diluting. Dilute 1 mL of serum with 1 mL of saline for a dilution of ½, or 1:2. Pipette off 1 mL of this dilution into an aliquot tube. Add 1 mL of saline, and it becomes a 1:4 dilution. If you dilute up to 1:32 and get no reaction, the end-point titer is 16.

30. A: Guinea pig, cow, and horse. Monospot heterophile antibodies test confirms an early infection of mononucleosis, caused by Epstein-Barr virus. If the infection is older than 9 weeks, then the doctor orders EBV antibody test. On a glass slide, mix a drop of the patient's blood with guinea pig kidney antigen to absorb Forssman antibodies. Add beef red blood stroma to absorb non-Forssman antibodies. Mix with horse blood. Guinea pig agglutination means the patient has early mononucleosis. Beef should not agglutinate. Monospot can be false-negative on children younger than 10, or before two weeks of infection. B, C, and D are not applicable to Monospot.

31. C: Both syphilis and HIV. Patients coinfecting with HIV and syphilis are immunosuppressed. When performing a titer to find antibodies in an HIV/syphilitic, beware prozone phenomenon. The coinfecting patient's undiluted serum may produce a false-negative result because it does not agglutinate. Alternatively, it may show very little agglutination at low dilutions, but agglutinates more at higher dilutions because of excess antibodies. Monospot is used to find EBV mononucleosis. Reynaud disease is characterized by rouleaux formation and high cold agglutinin titers. IgG occurs in patients who are convalescing from mononucleosis.

32. D: Both a and b. *RhoGAM* is the brand name for Rh immunoglobulin. It is administered to Rh- women who acquired anti-D antibodies from a previous blood transfusion or pregnancy. The infant and father do not receive *RhoGAM* at all. If there is a live birth, the mother gets 300 mcg of *RhoGAM* during week 26 to 28 of her pregnancy, and again before her infant is 3 days old. If the pregnancy miscarries before week 13 or is aborted, then the mother gets a lower dose of 50 mcg of *MICRhoGAM*. If the miscarriage or abortion happens after week 13, use *RhoGAM*.
33. A: Eosinophilia, hypocalcemia, leukopenia, and pancytopenia may occur. The first lab sign of a mild transfusion reaction is the oxyhemoglobin dissociation curve shifts left. Later, the number of eosinophils will increase and the calcium level will drop. Finally, white blood cells will decrease, and then all blood cells will decrease. Minimize the chance of transfusion reaction by washing the donor's red blood cells in sterile normal saline before transfusion. If the doctor anticipates a mild transfusion reaction, he/she may give antihistamines to the patient before transfusion, and may order the removal of white cells from the bag of blood by a Sepacell R-500 leukocyte reduction filter. Irradiated blood products prevent fatal transfusion-associated graft-versus-host disease (TA-GVHD). The safest way for a patient to prepare for elective surgery is to bank his own blood for transfusion (autologous donation).
34. C: No A or B antigens and no anti-A or anti-B antibodies. Type O- blood is the universal donor because it has no A or B antigens or anti-A or anti-B antibodies, or Rh+ antibodies. If there is no time to crossmatch a trauma patient, then O- blood is given without compatibility testing to prevent death. A routine type and cross takes 45 minutes and the delay could be fatal.
35. B: Trauma patient with hemoglobin of 5 g/dL. Blood Bank triages patients in the following priority sequence: (1) emergency trauma victims with isovolemic anemia from hemorrhage; (2) surgical patients who lose more than 3 cups of blood; (3) regular users of coagulation factors. If you anticipate a blood shortage because of a massive trauma, then contact the nurse manager as soon as possible. The surgical team may decide to cancel elective surgery, or delay it until the patient is medically treated to reduce anemia. If surgery must proceed, the surgical team may consider the following blood conservation methods if you warn them ahead of time: erythropoietin, autologous donations, or hemodilution before surgery; cell savers, hypotension, electrocautery, and lasers during surgery; and administering antifibrinolytics after surgery.

36. D: All of the above. A type and cross is very time-consuming (45 minutes) and must meet very specific safety standards to avoid a transfusion reaction. All of the following conditions must be met:
- Specimens labeled at the patient's bedside with full name or the emergency department identification number; initials are unacceptable. Specimens must not have pink serum. Donor blood must not be clotted, fatty, or contaminated.
  - Patient wears an identification band, which is checked at collection *and* transfusion times. The band must not be taped to the bed. The patient's name and a unique identification number (Blood Bank identification number, hospital number, health insurance number, or unique lifetime identifier) must appear on the band, in case there is a patient with a similar name.
  - Requisitions must bear the collector's and identifier's names, collection date and time (in case antibodies develop), the ordering doctor's name, the amount and type of blood requested, the patient's date of birth (if known), relevant patient history (e.g., pregnant and bleeding; signs of transfusion reaction).
37. A: Midstream urine collection (MSU). MSU is required to diagnose cystitis and pyelitis accurately. Witnessed collection is only required for drug testing. 24-hour urines are for hormone tests. Random urine may have contamination, so while it is suitable for chemistry, random urine is inaccurate for microbiology. Collect midstream urine any time of day, in a sterile, lidded container. Your microbiologist may want the patient to use a benzalkonium chloride wipe before collection. Without a wipe, the sample is not a clean catch. Do not touch the inside of the container, as it contaminates the specimen and produces a false-positive.
38. A: Cell count, glucose and protein, gram stain and culture, virology/mycology/cytology. Only a physician can collect cerebrospinal fluid (CSF) from a lumbar puncture. The MT just prepares a collection tray and assists as ordered. The tray must contain the following: iodine prep; alcohol prep; 3 cc of 1% lidocaine; 25g, 5/8" needle; 22g, 1.5" needle; atraumatic spinal needle (to prevent postcollection headache); syringe; four sterile red stoppered tubes; 4x4 gauze; sponge forceps; sterile towels; small basin; and a Band-Aid. The physician collects the fluid between L3 and L4 in the patient's spine and hands you the tubes. Label one tube each for cell count, glucose and protein, gram stain and culture, and virology/mycology/cytology. You only need a fifth tube if the physician wants globulin immunoelectrophoresis, which is rare. C and D tests are included in A, and it is unnecessary to requisition them separately.
39. C: Pyorrhea and Lemierre syndrome. The pathogenic phyla are xenobacteria, cyanobacteria, firmicutes, flavobacteria, fusobacteria, planctomycetes, proteobacteria, spirochaetes, and verrucomicrobia. Planctomycetes causes chlamydia and pneumonia. Spirochaetes cause Lyme disease. Proteobacteria causes stomach ulcers. Firmicutes cause food poisoning. Fusobacteria cause pockets of pus in the gums that can break off into septic blood clots in the jugular vein of the neck. The septic clots can travel to cause abscesses in distant parts of the body, such as the brain, joints, kidney, and liver. Lemierre syndrome from gum disease was common until the discovery of antibiotics.

40. D.: Löwenstein-Jensen (LJ) egg. TB is a fussy bacterium to grow in the lab and requires egg media. Tinsdale is used to find *C. diphtheria*. Sheep blood is used to find slow-growing anaerobic bacteria. MYW is used to find *Legionella* pneumonia. It is important for the MT to know what type of infection the doctor suspects, so the correct media can be used for culture. Failure to pick the correct media may result in a false-negative and the disease will go undiagnosed.
41. B: 10x. To find parasites such as worms, set the microscope's magnification to 10x. Parasites often cause bleeding, so set the microscope to 40x to find the blood cells. Higher powers are unnecessary to view animal parasites and count cells, and would just slow down the MT's reading of the slide. Calibrate the ocular micrometer every time a new technician is hired, each time you change optics, and annually thereafter.
42. A: Crypto (*Cryptosporidium parvum*). In the United States, the lab technician is required by law to report the following nine parasites to Public Health authorities if they are found in patient samples: *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, hematoxylin, *Giardia duodenalis*, *Plasmodium falciparum*, *Taenia*, *Trichinella spiralis*, and *Enterobius vermicularis*. Hookworm, tapeworm, and pinworm are very common infestations and do not need to be reported. Your lab must provide reference slides or a parasite atlas for you to compare against the patients' specimens. Keep positive specimens in your lab for at least one year, either as a permanently stained slide, or as a preserved stool sample that is safely stored. Public Health may order them for examination.
43. D: Wet mount fresh, liquid stool with LPCB stain. *Giardia lamblia* is a parasite that lives in the small intestine of humans who consume contaminated food or water. *Giardia* causes traveler's diarrhea. *Giardia* cysts are activated by stomach acid and become trophozoites. The MT can get the patient to swallow a string for several hours and then examine it for trophozoites, but many patients are uncooperative and prefer to leave a stool sample instead. To prepare the wet mount, strain well-formed stool. Concentrate it in the centrifuge at 2000 rpm for 4 minutes in a conical tube. Ream the tube with a wooden stick. Add 10% formalin. Make a tan suspension. You should be able to read a newspaper through the slide. Examine microscopically at 10x for parasites and 40x for blood. Use an ocular micrometer to measure parasites. Mix stool with PVA plastic powder to glue it onto the slide before permanent staining with iodine or *Snap n' Stain*.
44. B: One year. Parasites are a serious Public Health issue. It is important to prevent parasites acquired in foreign countries from spreading through the American populace. Even though you check your patient's specimen against reference slides or a parasite atlas, you could miss rare species or misidentify the parasite in its different stages of development. A Public Health official has the right to check your slide for one year after initial testing. To ensure your test is accurate, use positive and negative controls to check your antigens every time you receive a new shipment and every month thereafter. Use the right stain for the right specimen. Refrigerate stool within three hours of receiving it, if you do not have time to fix it with preservative.

45. D: Mycology specimens. The MT uses a Wood's lamp to help identify fungus on the patient's skin before collecting it. Fungus will fluoresce bright lime green under the Wood's light, so the MT will find it easily and can scrape it off with a tongue depressor into a sterile container for testing. Malaria parasites are found in blood smears. Public Health specimens are usually blood serology or stool for parasites. Toxicology specimens are usually red, navy, or purple stoppered blood tubes for drugs or heavy metals.
46. D: Nitrites. There is a nitrite pad on an *N-Multistix* for routine urinalysis. Some bacteria eat nitrates in urine, and shed nitrites as a waste product. *E. coli* is one of the bacteria detectable by nitrites in urine. However, not finding nitrites does not mean there is no infection. For example, strep is a very serious and common kidney infection, but does not produce nitrites. Leukocytes indicate an infection in a person with a healthy immune system, but an immunosuppressed patient may not produce leukocytes in urine. Protein indicates kidney damage, but it is not necessarily from an infection. Ketones are a byproduct of fat metabolism that appear in diabetic patients and crash dieters.
47. A: Testicular cancer. Do not assume that the doctor filled out the wrong name and sex on the requisition if you receive a request for a pregnancy test on a man. A pregnancy test looks for the hormone beta-human chorionic gonadotropin (hCG). A pregnant woman excretes beta-hCG 10 days after conceiving a child. However, a male with carcinoma of the testicles also excretes this same pregnancy hormone. Normal males never excrete beta-hCG. If your patient had an orchiectomy (removal of the testicles) but is still excreting beta-hCG in a follow-up test, then metastasized cancer is present. The doctor must remove it surgically, or with radiation or chemotherapy. Prostatitis would produce leukocytes but not hCG. Cryptorchidism is undescended testicles, which can lead to cancer in later life. Peyronie disease is a bent penis from scar tissue, and does not produce hCG.
48. D: 750 to 2,000 mL. A patient should produce at least 500 mL (2 cups) of urine each and every day. Ideally, a patient should produce 750 mL (3 cups) to 2,000 mL (5 cups) of urine to maintain good health. If the patient has vomiting and diarrhea, or an enlarged prostate gland or severe infection, or uses too much medication, then he will produce scanty urine (oliguria). Some of the drug overdoses that decrease urinary output are anticholinergics, methotrexate, and diuretics. Patients whose kidneys are failing have anuria, which strictly interpreted means absence of urine, but they actually produce 100 mL or less of urine per day. Patients who have diabetes insipidus or diabetes mellitus often produce far too much urine (3½ quarts or more). They are very thirsty and may drink more than a gallon of fluid per day (more than 12 glasses). The antidepressant lithium is one drug that can cause frequent urination as an adverse effect.
49. C: Gout. Patients with gout have extreme pain in their great toes due to needles of uric acid crystals that form around their joints. Patients with struvite crystals in their urine have bacterial infections. Patients with tyrosine or cystine crystals in their urine may be

poisoned or have a serious metabolic disorder. Patients with phosphate or calcium oxalate crystals in their urine have too much parathyroid hormone or malabsorption. Crystals do not appear in healthy urine.

50. C: Hematuria and uroliths. Polycystic kidney disease is an inherited disorder that produces bloody urine and kidney stones. It is the most common inherited disease in the United States, and it is the fourth leading cause of kidney failure. Cysts may also appear in the patient's liver, but do not produce cirrhosis or hepatitis. Destruction of the kidney does eventually produce high blood pressure, but the PKD patient is a poor candidate for a bypass graft because of abnormal heart valves. The patient may develop abnormal pockets in the intestine that fill with hard stool (diverticulosis); however, they seldom produce severe bleeding. Melena is a tarry, black stool caused by internal bleeding, and hematochezia is red stools caused by heavy bleeding.

## Secret Key #1 - Time is Your Greatest Enemy

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### *Pace Yourself*

Wear a watch. At the beginning of the test, check the time (or start a chronometer on your watch to count the minutes), and check the time after every few questions to make sure you are “on schedule.”

If you are forced to speed up, do it efficiently. Usually one or more answer choices can be eliminated without too much difficulty. Above all, don't panic. Don't speed up and just begin guessing at random choices. By pacing yourself, and continually monitoring your progress against your watch, you will always know exactly how far ahead or behind you are with your available time. If you find that you are one minute behind on the test, don't skip one question without spending any time on it, just to catch back up. Take 15 fewer seconds on the next four questions, and after four questions you'll have caught back up. Once you catch back up, you can continue working each problem at your normal pace.

Furthermore, don't dwell on the problems that you were rushed on. If a problem was taking up too much time and you made a hurried guess, it must be difficult. The difficult questions are the ones you are most likely to miss anyway, so it isn't a big loss. It is better to end with more time than you need than to run out of time.

Lastly, sometimes it is beneficial to slow down if you are constantly getting ahead of time. You are always more likely to catch a careless mistake by working more slowly than quickly, and among very high-scoring test takers (those who are likely to have lots of time left over), careless errors affect the score more than mastery of material.



## Secret Key #2 - Guessing is not Guesswork

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You probably know that guessing is a good idea - unlike other standardized tests, there is no penalty for getting a wrong answer. Even if you have no idea about a question, you still have a 20-25% chance of getting it right.

Most test takers do not understand the impact that proper guessing can have on their score. Unless you score extremely high, guessing will significantly contribute to your final score.

### *Monkeys Take the Test*

What most test takers don't realize is that to insure that 20-25% chance, you have to guess randomly. If you put 20 monkeys in a room to take this test, assuming they answered once per question and behaved themselves, on average they would get 20-25% of the questions correct. Put 20 test takers in the room, and the average will be much lower among guessed questions. Why?

1. The test writers intentionally write deceptive answer choices that "look" right. A test taker has no idea about a question, so picks the "best looking" answer, which is often wrong. The monkey has no idea what looks good and what doesn't, so will consistently be lucky about 20-25% of the time.
2. Test takers will eliminate answer choices from the guessing pool based on a hunch or intuition. Simple but correct answers often get excluded, leaving a 0% chance of being correct. The monkey has no clue, and often gets lucky with the best choice.

This is why the process of elimination endorsed by most test courses is flawed and detrimental to your performance- test takers don't guess, they make an ignorant stab in the dark that is usually worse than random.

## \$5 Challenge

Let me introduce one of the most valuable ideas of this course- the \$5 challenge:

*You only mark your "best guess" if you are willing to bet \$5 on it.*

*You only eliminate choices from guessing if you are willing to bet \$5 on it.*

Why \$5? Five dollars is an amount of money that is small yet not insignificant, and can really add up fast (20 questions could cost you \$100). Likewise, each answer choice on one question of the test will have a small impact on your overall score, but it can really add up to a lot of points in the end.

The process of elimination IS valuable. The following shows your chance of guessing it right:

If you eliminate wrong answer choices until only this many remain:	1	2	3
Chance of getting it correct:	100%	50%	33%

However, if you accidentally eliminate the right answer or go on a hunch for an incorrect answer, your chances drop dramatically: to 0%. By guessing among all the answer choices, you are GUARANTEED to have a shot at the right answer.

That's why the \$5 test is so valuable- if you give up the advantage and safety of a pure guess, it had better be worth the risk.

What we still haven't covered is how to be sure that whatever guess you make is truly random. Here's the easiest way:

*Always pick the first answer choice among those remaining.*

Such a technique means that you have decided, **before you see a single test question**, exactly how you are going to guess- and since the order of choices tells you nothing about which one is correct, this guessing technique is perfectly random.

This section is not meant to scare you away from making educated guesses or eliminating choices- you just need to define when a choice is worth eliminating. The \$5 test, along with a pre-defined random guessing strategy, is the best way to make sure you reap all of the benefits of guessing.

## Secret Key #3 - Practice Smarter, Not Harder

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Many test takers delay the test preparation process because they dread the awful amounts of practice time they think necessary to succeed on the test. We have refined an effective method that will take you only a fraction of the time.

There are a number of “obstacles” in your way to succeed. Among these are answering questions, finishing in time, and mastering test-taking strategies. All must be executed on the day of the test at peak performance, or your score will suffer. The test is a mental marathon that has a large impact on your future.

Just like a marathon runner, it is important to work your way up to the full challenge. So first you just worry about questions, and then time, and finally strategy:

### *Success Strategy*

1. Find a good source for practice tests.
2. If you are willing to make a larger time investment, consider using more than one study guide- often the different approaches of multiple authors will help you “get” difficult concepts.
3. Take a practice test with no time constraints, with all study helps “open book.” Take your time with questions and focus on applying strategies.
4. Take a practice test with time constraints, with all guides “open book.”
5. Take a final practice test with no open material and time limits

If you have time to take more practice tests, just repeat step 5. By gradually exposing yourself to the full rigors of the test environment, you will condition your mind to the stress of test day and maximize your success.

## Secret Key #4 - Prepare, Don't Procrastinate

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Let me state an obvious fact: if you take the test three times, you will get three different scores. This is due to the way you feel on test day, the level of preparedness you have, and, despite the test writers' claims to the contrary, some tests WILL be easier for you than others.

Since your future depends so much on your score, you should maximize your chances of success. In order to maximize the likelihood of success, you've got to prepare in advance. This means taking practice tests and spending time learning the information and test taking strategies you will need to succeed.

Never take the test as a "practice" test, expecting that you can just take it again if you need to. Feel free to take sample tests on your own, but when you go to take the official test, be prepared, be focused, and do your best the first time!

## Secret Key #5 - Test Yourself

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Everyone knows that time is money. There is no need to spend too much of your time or too little of your time preparing for the test. You should only spend as much of your precious time preparing as is necessary for you to get the score you need.

Once you have taken a practice test under real conditions of time constraints, then you will know if you are ready for the test or not.

If you have scored extremely high the first time that you take the practice test, then there is not much point in spending countless hours studying. You are already there.

Benchmark your abilities by retaking practice tests and seeing how much you have improved. Once you score high enough to guarantee success, then you are ready.

If you have scored well below where you need, then knuckle down and begin studying in earnest. Check your improvement regularly through the use of practice tests under real conditions. Above all, don't worry, panic, or give up. The key is perseverance!

Then, when you go to take the test, remain confident and remember how well you did on the practice tests. If you can score high enough on a practice test, then you can do the same on the real thing.

# General Strategies

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The most important thing you can do is to ignore your fears and jump into the test immediately- do not be overwhelmed by any strange-sounding terms. You have to jump into the test like jumping into a pool- all at once is the easiest way.

## **Make Predictions**

As you read and understand the question, try to guess what the answer will be. Remember that several of the answer choices are wrong, and once you begin reading them, your mind will immediately become cluttered with answer choices designed to throw you off. Your mind is typically the most focused immediately after you have read the question and digested its contents. If you can, try to predict what the correct answer will be. You may be surprised at what you can predict.

Quickly scan the choices and see if your prediction is in the listed answer choices. If it is, then you can be quite confident that you have the right answer. It still won't hurt to check the other answer choices, but most of the time, you've got it!

## **Answer the Question**

It may seem obvious to only pick answer choices that answer the question, but the test writers can create some excellent answer choices that are wrong. Don't pick an answer just because it sounds right, or you believe it to be true. It **MUST** answer the question. Once you've made your selection, always go back and check it against the question and make sure that you didn't misread the question, and the answer choice does answer the question posed.

## **Benchmark**

After you read the first answer choice, decide if you think it sounds correct or not. If it doesn't, move on to the next answer choice. If it does, mentally mark that answer choice. This doesn't mean that you've definitely selected it as your answer choice, it just means that it's the best you've seen thus far. Go ahead and read the next choice. If the next choice is worse than the one you've already selected, keep going to the next answer choice. If the next choice is better than the choice you've already selected, mentally mark the new answer choice as your best guess.

The first answer choice that you select becomes your standard. Every other answer choice must be benchmarked against that standard. That choice is correct until proven otherwise by another answer choice beating it out. Once you've decided that no other answer choice seems as good, do one final check to ensure that your answer choice answers the question posed.

## **Valid Information**

Don't discount any of the information provided in the question. Every piece of information may be necessary to determine the correct answer. None of the information in the question is there to throw you off (while the answer choices will certainly have information to throw you off). If two seemingly unrelated topics are discussed, don't ignore either. You can be confident there is a relationship, or it wouldn't be included in the question, and you are probably going to have to determine what is that relationship to find the answer.

## **Avoid "Fact Traps"**

Don't get distracted by a choice that is factually true. Your search is for the answer that answers the question. Stay focused and don't fall for an answer that is true but incorrect. Always go back to the question and make sure you're choosing an answer that actually answers the question and is not just a true statement. An answer can be factually correct, but it **MUST** answer the question asked. Additionally, two answers can both be seemingly correct, so be sure to read all of the answer choices, and make sure that you get the one that **BEST** answers the question.

## **Milk the Question**

Some of the questions may throw you completely off. They might deal with a subject you have not been exposed to, or one that you haven't reviewed in years. While your lack of knowledge about the subject will be a hindrance, the question itself can give you many clues that will help you find the correct answer. Read the question carefully and look for clues. Watch particularly for adjectives and nouns describing difficult terms or words that you don't recognize. Regardless of if you completely understand a word or not, replacing it with a synonym either provided or one you more familiar with may help you to understand what the questions are asking. Rather than wracking your mind about specific detailed information concerning a difficult term or word, try to use mental substitutes that are easier to understand.

## **The Trap of Familiarity**

Don't just choose a word because you recognize it. On difficult questions, you may not recognize a number of words in the answer choices. The test writers don't put "make-believe" words on the test; so don't think that just because you only recognize all the words in one answer choice means that answer choice must be correct. If you only recognize words in one answer choice, then focus on that one. Is it correct? Try your best to determine if it is correct. If it is, that is great, but if it doesn't, eliminate it. Each word and answer choice you eliminate increases your chances of getting the question correct, even if you then have to guess among the unfamiliar choices.

## **Eliminate Answers**

Eliminate choices as soon as you realize they are wrong. But be careful! Make sure you consider all of the possible answer choices. Just because one appears right, doesn't mean that the next one won't be even better! The test writers will usually put more than one good answer choice for every question, so read all of them. Don't worry if you are stuck

between two that seem right. By getting down to just two remaining possible choices, your odds are now 50/50. Rather than wasting too much time, play the odds. You are guessing, but guessing wisely, because you've been able to knock out some of the answer choices that you know are wrong. If you are eliminating choices and realize that the last answer choice you are left with is also obviously wrong, don't panic. Start over and consider each choice again. There may easily be something that you missed the first time and will realize on the second pass.

## **Tough Questions**

If you are stumped on a problem or it appears too hard or too difficult, don't waste time. Move on! Remember though, if you can quickly check for obviously incorrect answer choices, your chances of guessing correctly are greatly improved. Before you completely give up, at least try to knock out a couple of possible answers. Eliminate what you can and then guess at the remaining answer choices before moving on.

## **Brainstorm**

If you get stuck on a difficult question, spend a few seconds quickly brainstorming. Run through the complete list of possible answer choices. Look at each choice and ask yourself, "Could this answer the question satisfactorily?" Go through each answer choice and consider it independently of the other. By systematically going through all possibilities, you may find something that you would otherwise overlook. Remember that when you get stuck, it's important to try to keep moving.

## **Read Carefully**

Understand the problem. Read the question and answer choices carefully. Don't miss the question because you misread the terms. You have plenty of time to read each question thoroughly and make sure you understand what is being asked. Yet a happy medium must be attained, so don't waste too much time. You must read carefully, but efficiently.

## **Face Value**

When in doubt, use common sense. Always accept the situation in the problem at face value. Don't read too much into it. These problems will not require you to make huge leaps of logic. The test writers aren't trying to throw you off with a cheap trick. If you have to go beyond creativity and make a leap of logic in order to have an answer choice answer the question, then you should look at the other answer choices. Don't overcomplicate the problem by creating theoretical relationships or explanations that will warp time or space. These are normal problems rooted in reality. It's just that the applicable relationship or explanation may not be readily apparent and you have to figure things out. Use your common sense to interpret anything that isn't clear.

## **Prefixes**

If you're having trouble with a word in the question or answer choices, try dissecting it. Take advantage of every clue that the word might include. Prefixes and suffixes can be a huge help. Usually they allow you to determine a basic meaning. Pre- means before, post- means after, pro - is positive, de- is negative. From these prefixes and suffixes, you can get



an idea of the general meaning of the word and try to put it into context. Beware though of any traps. Just because con is the opposite of pro, doesn't necessarily mean congress is the opposite of progress!

### **Hedge Phrases**

Watch out for critical "hedge" phrases, such as likely, may, can, will often, sometimes, often, almost, mostly, usually, generally, rarely, sometimes. Question writers insert these hedge phrases to cover every possibility. Often an answer choice will be wrong simply because it leaves no room for exception. Avoid answer choices that have definitive words like "exactly," and "always".

### **Switchback Words**

Stay alert for "switchbacks". These are the words and phrases frequently used to alert you to shifts in thought. The most common switchback word is "but". Others include although, however, nevertheless, on the other hand, even though, while, in spite of, despite, regardless of.

### **New Information**

Correct answer choices will rarely have completely new information included. Answer choices typically are straightforward reflections of the material asked about and will directly relate to the question. If a new piece of information is included in an answer choice that doesn't even seem to relate to the topic being asked about, then that answer choice is likely incorrect. All of the information needed to answer the question is usually provided for you, and so you should not have to make guesses that are unsupported or choose answer choices that require unknown information that cannot be reasoned on its own.

### **Time Management**

On technical questions, don't get lost on the technical terms. Don't spend too much time on any one question. If you don't know what a term means, then since you don't have a dictionary, odds are you aren't going to get much further. You should immediately recognize terms as whether or not you know them. If you don't, work with the other clues that you have, the other answer choices and terms provided, but don't waste too much time trying to figure out a difficult term.

### **Contextual Clues**

Look for contextual clues. An answer can be right but not correct. The contextual clues will help you find the answer that is most right and is correct. Understand the context in which a phrase or statement is made. This will help you make important distinctions.

### **Don't Panic**

Panicking will not answer any questions for you. Therefore, it isn't helpful. When you first see the question, if your mind goes blank, take a deep breath. Force yourself to mechanically go through the steps of solving the problem and using the strategies you've learned.

## **Pace Yourself**

Don't get clock fever. It's easy to be overwhelmed when you're looking at a page full of questions, your mind is full of random thoughts and feeling confused, and the clock is ticking down faster than you would like. Calm down and maintain the pace that you have set for yourself. As long as you are on track by monitoring your pace, you are guaranteed to have enough time for yourself. When you get to the last few minutes of the test, it may seem like you won't have enough time left, but if you only have as many questions as you should have left at that point, then you're right on track!

## **Answer Selection**

The best way to pick an answer choice is to eliminate all of those that are wrong, until only one is left and confirm that is the correct answer. Sometimes though, an answer choice may immediately look right. Be careful! Take a second to make sure that the other choices are not equally obvious. Don't make a hasty mistake. There are only two times that you should stop before checking other answers. First is when you are positive that the answer choice you have selected is correct. Second is when time is almost out and you have to make a quick guess!

## **Check Your Work**

Since you will probably not know every term listed and the answer to every question, it is important that you get credit for the ones that you do know. Don't miss any questions through careless mistakes. If at all possible, try to take a second to look back over your answer selection and make sure you've selected the correct answer choice and haven't made a costly careless mistake (such as marking an answer choice that you didn't mean to mark). This quick double check should more than pay for itself in caught mistakes for the time it costs.

## **Beware of Directly Quoted Answers**

Sometimes an answer choice will repeat word for word a portion of the question or reference section. However, beware of such exact duplication – it may be a trap! More than likely, the correct choice will paraphrase or summarize a point, rather than being exactly the same wording.

## **Slang**

Scientific sounding answers are better than slang ones. An answer choice that begins "To compare the outcomes..." is much more likely to be correct than one that begins "Because some people insisted..."

## **Extreme Statements**

Avoid wild answers that throw out highly controversial ideas that are proclaimed as established fact. An answer choice that states the "process should be used in certain situations, if..." is much more likely to be correct than one that states the "process should be discontinued completely." The first is a calm rational statement and doesn't even make

a definitive, uncompromising stance, using a hedge word “if” to provide wiggle room, whereas the second choice is a radical idea and far more extreme.

### **Answer Choice Families**

When you have two or more answer choices that are direct opposites or parallels, one of them is usually the correct answer. For instance, if one answer choice states “x increases” and another answer choice states “x decreases” or “y increases,” then those two or three answer choices are very similar in construction and fall into the same family of answer choices. A family of answer choices is when two or three answer choices are very similar in construction, and yet often have a directly opposite meaning. Usually the correct answer choice will be in that family of answer choices. The “odd man out” or answer choice that doesn’t seem to fit the parallel construction of the other answer choices is more likely to be incorrect.

## Special Report: Additional Bonus Material

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Due to our efforts to try to keep this book to a manageable length, we've created a link that will give you access to all of your additional bonus material.

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